Identification of vaccine candidates against the Poultry Red Mite, *Dermanyssus gallinae*

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Declaration:

I declare that this thesis has been entirely composed and the experimental work was carried out by myself, except where clearly stated otherwise.

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Abbreviations:

APCs	Antigen presenting cells
BLAST	Basic linear alignment search tool
BSA	Bovine serum albumen
Blo t	*Blomia tropicalis*
cDNA	Complementary deoxyribonucleic acid
COG	Cluster of Orthologous Proteins
Contig	Contigous sequence
cv	column volumes
DAB	3,3'-Diaminobenzidine,
Der p	*Dermatophagoides pteronyssinus*
Der f	*Dermatophagoides farinae*
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EST	Expressed sequence tag
Exo1	Exonuclease 1
Gbp	Gigabasepairs
GLMM	Generalised linear mixed model
GST	Glutathione S-transferase
HDM	House dust mite
HRP	Horse radish peroxidase
Ig	Immunoglobulin
IPTG	Isopropyl-β-D-thiogalactopyranoside
kbp	Kilo-base pairs
KCl	Potassium chloride
kDa	Kilo-daltons
LMM	Linear mixed model
LB	Luria Bertani
LBG	Luria Bertani glycerol
MAC	Membrane attack complex
MALDI –TOF	Matrix assisted laser desorption /ionization- Time of flight
MHC	Major histocompatibility complex
MS/MS	Mass Spectrometer/Mass Spectrometer
MTC	Multiple test correction
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
NGS	Normal goat serum

OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PMF	Peptide mass fingerprint
ppm	Parts per million
REML	Residual maximum likelihood
RNA	Ribonucleic acid
RT	Room temperature
RT-PCR	Real time PCR
SAP	Shrimp alkaline phosphotase
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
SMART	Switching mechanism at 5' end of RNA transcript
SSH	Suppression subtractive hybridisation
STP	Squalan, tween 80, pluronic
TAE	Tris-acetate-EDTA
TBS	Tris buffered saline
TBST	Tris buffered saline tween 20
TCR	T cell receptor
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
Tm	Melting temperature
u	Units of activity
WME	Whole mite extract

Abstract:

The poultry red mite *Dermanyssus gallinae* (De Geer) is a blood feeding ectoparasite that infests many bird species. Economically it is the most important parasite affecting egg-laying hens. The aim of this study was to identify vaccine candidate proteins from *D. gallinae* using a number of approaches.

An immunisation trial was conducted using four protein fractions derived from *D. gallinae*. The fractions were injected into hens and immunoglobulin Y was purified from the yolk of eggs laid by the hens. An *in vitro* feeding assay in which mites were fed these antibodies showed a significant increase ($p = 0.013$) in mortality of mites fed with antibodies against a PBS-soluble fraction of *D. gallinae*. The PBS-soluble proteins were then fractionated by anion exchange chromatography into three fractions, which were then used to immunise hens. One fraction produced antibodies that caused the greatest percentage mortality of mites, based on the results from a single feeding assay.

To identify further potential vaccine antigens, three expressed sequence tag (EST) databases were produced. A complementary DNA (cDNA) library was prepared from a mixed gender and life stage population and 46 contigs were identified with significant homology to proteins from other organisms. A second database was generated using a suppression subtractive hybridisation approach and 133 “fed mite enriched” contigs identified with significant homology to proteins in either the NCBI non-redundant (nr) protein, KEGG databases or had a domain predicted by InterProScan. The most abundant proteins in this database were vitellogenin and GP80, a precursor molecule of vitellogenin. Roche 454 sequencing technology was used to generate an EST database of 13,363 contigs, of which 26 % had significant homology to a protein in the NCBI nr protein database. The majority of contigs (45 %) were classified as Cellular Processes and Signalling proteins. Illumina Solexa sequencing technology was also used to analyse the expression levels of genes in fed and starved mites. A total of 66 contigs were obtained with a significant and greater than three-fold change in expression level between the two groups. The contig with the largest fold change was homologous to vitellogenin (fold change 110).

Paramyosin and tropomyosin have been used successfully to immunise hosts against other parasitic species. Tropomyosin had previously been characterised in *D. gallinae*. In this study paramyosin was characterised and recombinant versions of both proteins were used in an immunisation trial. Anti-tropomyosin ($p < 0.001$) and anti-paramyosin ($p = 0.004$) antibodies fed to mites in the *in vitro* feeding assay caused a significant increase in the mortality of the mites when compared to controls.

An *in vivo* challenge was performed injecting three groups of hens with the PBS-soluble native protein fraction, a recombinant protein cocktail, consisting of paramyosin, tropomyosin, cathepsin L, cathepsin D and histamine release factor, and an adjuvant only control, which were subjected to a challenge of *D. gallinae*. Each group consisted of three replicates based on weight. Western blot analysis of hen serum showed a significant increase ($p < 0.05$) in the titre of antibody from the hens immunised with the recombinant cocktail compared with controls. One of the replicates immunised with the PBS-soluble protein fraction showed a strong response but this was lacking in the other two replicates and no significant difference in IgY titre was found. No significant differences were found between the number of mites collected from the test or control groups following a large mite challenge.

In conclusion the PBS-soluble protein fraction, paramyosin and tropomyosin have been shown to have potential as vaccine candidates based on *in vitro* studies. The bioinformatic analysis of *D. gallinae* has provided a large EST database and a list of 66 proteins that had a significant difference in expression levels in fed and starved mites, which can be further mined for potential vaccine candidates.

Chapter 1:

General Introduction

1.1 Poultry farming

The rearing of domestic fowl is a huge industry worldwide. The scale of operation ranges from individuals who keep only a few free range hens to mass production by poultry farmers who keep hundreds of thousands of hens in battery cage, barnyard or free range systems where the eggs and meat produced are sold to supermarkets. The loss of production due to illness whether viral, bacterial or parasitic in nature is an important economic and welfare issue that needs to be continually addressed.

In the United Kingdom there are 29.1 million laying hens, which makes the UK the sixth largest egg producer in Europe (British Egg Industry Council, 2008). The eggs are classified into two distinct categories depending on whether the hens are housed in either battery cages or non-caged systems. Non-caged systems can be classified as free range, barnyard housed or organic.

Hens kept for meat or egg production are stocked at high densities, which can exacerbate the spread of disease throughout a flock. Each of the housing systems (discussed in 1.2) can be infested with the poultry red mite, *Dermanyssus gallinae*, and the levels of mites present are related to increases in hen mortality (Arkle et al., 2006). Prevalence studies in England have shown that *D. gallinae* infestations are higher in the barnyard system than in battery cages (Fiddes et al., 2005). Historically, chemicals have been used to control *D. gallinae*. However, since the deregulation of some of the most effective chemicals that cause a build up of toxic residues in food products (Hamscher et al., 2003), an alternative control method is required. In 2005, the estimated cost incurred by *D. gallinae* to the European Union was 130 million Euros (van Emous R, 2005).

1.2 *Dermanyssus gallinae*

Dermanyssus gallinae (de Geer, 1778) is a haematophagous ectoparasite that infests many bird species worldwide. It is also known as the poultry red mite due to its preference for feeding on poultry and the red colouration of its body after feeding. Figure 1.1 is a photograph of an adult mite.

Figure 1.1. Adult *D. gallinae* after a blood meal.

All of the systems for poultry farming as described in 1.3 have problems with *D. gallinae* infestation. From an economic point of view it is one of the most important ectoparasites affecting egg-laying hens in many countries worldwide (Chauve, 1998). A survey carried out in Northern England revealed that 87.5 % of the 17 farms surveyed were infested with *D. gallinae*. Infestation was confirmed by setting traps near the hens and counting the number of mites collected after seven days (Guy et al., 2004). A similar survey in Sweden revealed that 67 % of the 21 farms that responded had *D. gallinae* infestation in backyard flocks. Infestation was confirmed by inspections, sampling and interviewing the farmers (Hoglund et al., 1995). As described in 1.2.3 *D. gallinae* affects the egg-laying potential of the hens due to blood loss and irritability. However, the main cause of economic egg loss due to mite infestation is cosmetic in nature. In a battery cage farm, when eggs are laid, they roll out of the cage onto a conveyer belt, where they cast a shadow. The mites

make use of this darkened area to hide. When the conveyer belt is activated the eggs roll backwards slightly and crush the blood-engorged mites thereby forming a blood spot on the eggs. Eggs with these blood spots cannot be sold on cosmetic grounds and have to be washed. After the eggs have been washed they are downgraded in quality and therefore value.

D. gallinae rests during the day in any dark place, collecting in cracks and crevices provided by nest boxes or machinery in the poultry housing. Mites infest hens during the night and feed for approximately 90 minutes. They then retreat to the dark hiding places to digest the blood meal after which they moult, reproduce and lay eggs. Experiments conducted to study the longevity of *D. gallinae* found that the mites can live for at least eight months in natural conditions in a poultry house that has been depopulated of hens. During the experiment, which ran for nine months over the winter to the beginning of summer, the temperature ranged from -10°C to 32.7°C. The mite population dramatically decreased in June (month eight), when the temperature was at its highest, to 56 mites from hundreds observed at the end of April. No mites were found during a careful search of the poultry house at the end of July (month nine) and since only a few dead mites were found in the hen house at this time it was concluded that the mites had migrated in search of a blood meal (Kirkwood, 1963). The effects of temperature and humidity on oviposition, moulting and longevity of *D. gallinae* from a Swedish poultry farm were tested. Mites were collected from a poultry farm and exposed to a range of temperatures from -20°C to 65°C in multi-well plates for 41 weeks. *D. gallinae* can survive in locations where the temperature is moderate, between 5°C and 45°C with female mites laying the greatest number of eggs at 25°C but do not tolerate temperature extremes, below -20°C and above 45°C. Mites were also exposed to a range of relative humidity from 30 % to 90 %. The highest number of eggs were laid at a relative humidity of 70 % (Nordenfors et al., 1999). These conditions are optimal for a mite to mature from an egg to a sexually mature adult in the shortest period of time. The ability of *D. gallinae* to survive for such long periods without a bloodmeal makes the de-infestation of barns and cages a problem that needs to be addressed before a new flock can be introduced. This is especially important in a commercial environment

where rapid turnover is a priority. In a more general study it was suggested that a minimum period of four weeks was required between housing separate flocks to allow the mite population to decrease to a manageable level (Beugnet et al., 1997).

D. gallinae have been found in poultry units all around the world (Kim et al., 2007; Roy & Chauve, 2009). Studies have been carried out in European countries to assess and control the populations of *D. gallinae* in poultry houses. In France massive infestations are common, where *D. gallinae* populations that are resistant to pyrethroid acaricides have been reported. The experiments were carried out *in vitro*, where mites were incubated for 24 hours on filter paper soaked in the chemical. The resistance detected in mites to these acaricides was reported to be similar to the resistance that developed in tick species, in particular *Rhipicephalus* (*Boophilus*) *microplus* (Beugnet et al., 1997). Surveys in Sweden, followed by visits to the farms were carried out to assess the prevalence of *D. gallinae* in the different housing systems. After inspection it was found that only 4 % of cage systems were infested, whereas as many as 67 % of backyard flocks and 33 % of barn or deep litter flocks were infested. The percentages for the backyard and barn or deep litter systems were much higher on inspection than was reported by farmers that responded to the survey, which stated that 19 % of backyard flocks and 21 % of barn or deep litter flocks were infested with mites. This suggested that either the situation had changed between the survey and the inspection or that the poultry workers could not identify mite infestation (Hoglund et al., 1995).

1.2.1 Taxonomy

By morphology, *Dermanyssus gallinae* is described as a member of the Phylum Arthropoda, Class Arachnida, Order Acari, Sub-order Mesostigmata and Family Dermanyssidae. After reclassification of the original 56 members of the genus *Dermanyssus*, 23 members remain. *D. gallinae* is widely distributed and has been identified around the world (Roy & Chauve, 2009)

Another mite that parasitizes hens is *Ornithonyssus sylviarum* (Northern Fowl Mite [NFM]) of the sub-order Mesostigmata and family Macronyssidae. The NFM is also a haematophagous ectoparasite of birds; however it spends its entire lifecycle on its host, which is most commonly poultry (Owen et al., 2009).

Studies were conducted to assess the phylogenetic relationship between populations of *D. gallinae* in Italy, France and the UK. The mitochondrial cytochrome oxidase I gene was sequenced from each of the populations sampled in the different countries. It was found that there was a high rate of nucleotide substitution in the gene between mite populations. When aligned, 51.5 % of the nucleotide sequence was conserved throughout the cytochrome oxidase I gene. The amino acid sequences were between 70 % and 87.9 % conserved within the samples from each country (Marangi et al., 2009a). This suggested that the majority of the non-conserved nucleotide sites were silent mutations. Genetic differences between *D. gallinae* populations throughout Europe and the rest of the world could result in naturally resistant strains of the mite to any possible vaccine.

1.2.2 Life cycle

The life cycle of *D. gallinae* has previously been described (Chauve, 1998; Wood, 1917). In brief, from egg to egg the entire life cycle can take as little as seven days. The six-legged larva will emerge from an egg laid three days previously. Within a day the larva will moult into an eight-legged protonymph. The protonymph takes its first blood meal and moults into a deutonymph within a day. The deutonymph feeds again and moults into an adult, which can then mate. After mating, the female feeds and starts to deposit eggs within 12 hours (Bruneau et al., 2001; Chauve, 1998). The female mite's laying life span is five to eight weeks, during which it can lay up to five clutches of about eight eggs (Meyer-Kuhling et al., 2007). In optimal conditions the mite population in a poultry house can double every six days (Maurer & Baumgartner, 1992). Figure 1.2 is an illustrated diagram of the red mite life cycle.

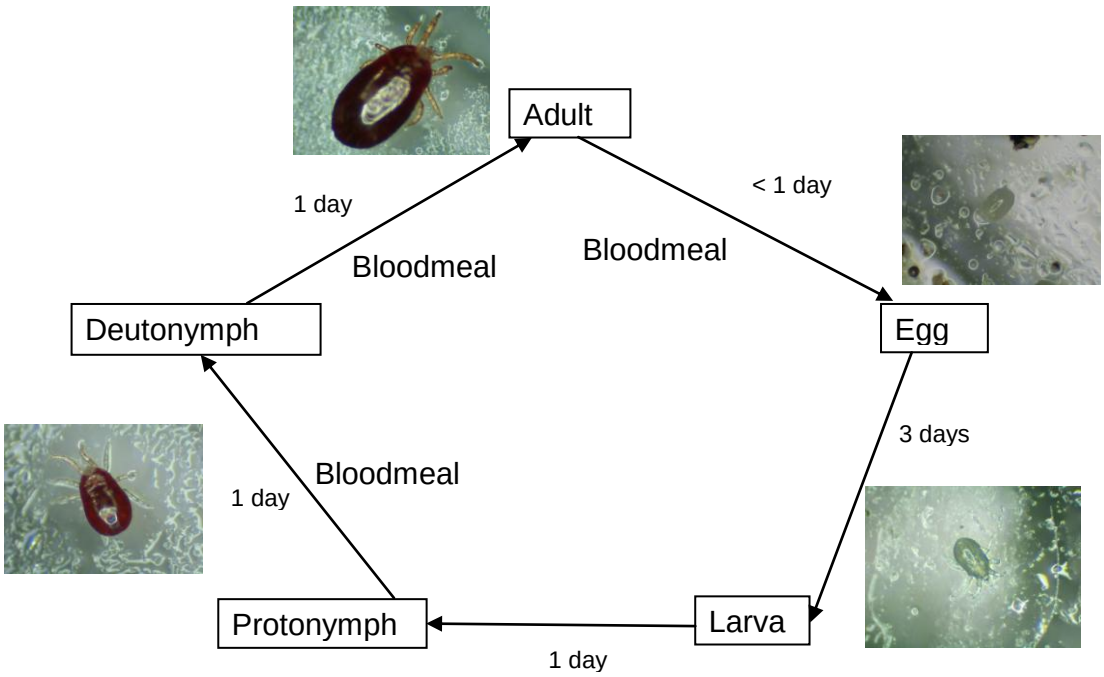


Figure 1.2. Life cycle of *D. gallinae*.

1.2.3 Clinical signs

Since *D. gallinae* is a haematophagous parasite, the main symptom in infested hens is anaemia. Due to the extremely high population of mites that can infest poultry houses, the hens can lose a large volume of blood per night. A single female mite has the ability to ingest an average blood meal of 204 µg (Sikes & Chamberlain, 1954) and in heavy infestations there may be as many as 200,000 mites infesting a single hen (Kilpinen et al., 2005). An obvious sign of anaemia in hens is loss of colour of the wattle and comb, which become pale pink instead of red.

Other clinical signs of heavily infested hens are changes in behaviour. At night the mites emerge from their hiding places to feed on the hens. This infestation leads to an increase in self grooming and social feather pecking (Kilpinen et al., 2005). The discomfort and itching caused by the feeding mites can deprive the hens of sleep. The resulting agitation leads to an increase in bullying by pecking, which causes excessive feather loss and physical damage to the weaker hens (Hughes & Duncan, 1972).

In extreme cases, anaemia and stress due to behavioural changes can result in death. However, the main effect of these symptoms is reduced egg-laying potential of the hens, which is one of the sources of economic loss suffered by farmers.

1.2.4 *D. gallinae* as a vector for disease

Many parasitic species are vectors for the mechanical and cyclical transmission of other pathogens, such as viruses and bacteria. *D. gallinae* has been shown to carry bacterium and therefore could be a vector in the mechanical transmission of several diseases that affect poultry. *D. gallinae* was collected from three farms on which the poultry had been diagnosed with hen erysipelas. Erysipelas is caused by the bacterium *Erysipelothrix rhusiopathiae*, which infects a number of vertebrate species including hens, pigs and humans. It causes an acute septicaemia in hens that can lead to death over a short period of time. The bacterium was present inside mites on all of the farms and was also found on the surface of the mites on one of the farms (Chirico et al., 2003). However, attempts to transmit the bacterium from infected *D.gallinae* to uninfected hens has not been successful (Brannstrom et al., 2010).

Salmonella spp. are important bacteria that infect but are non-pathogenic in poultry. *Salmonella* spp. can be passed on to humans though consumption of contaminated products such as eggs and undercooked meat and can cause diarrhoea, stomach cramps, vomiting and fever in humans. In severe cases dehydration may occur which can be life-threatening if not treated. *D. gallinae* is a reservoir for *Salmonella*, and is capable of passing the bacterium from hen to hen (Moro et al., 2007). Through the control of *D. gallinae* the spread of *Salmonella* spp. may be reduced.

1.2.5 Host specificity

The preferred host for *D. gallinae* is poultry. However, it will feed opportunistically on wild birds, domestic animals such as dogs and cats, and humans. Personnel working with birds or coming into close contact with them can develop severe

dermatitis even after short exposure to the parasite. A reaction to *D. gallinae* was found to be the responsible for two out of eight cases of pruritus during a study of human patients in Israel. In the first case, a group of seven male poultry workers reported seeing mites crawling over the eggs they were collecting. The second case was a young girl who had mites collected from her hair and from the wall next to her bed. An infested sparrow nest was found near her bedroom window (Rosen et al., 2002). Ectoparasites identified as *D. gallinae* were found on two patients with skin lesions in a Canadian hospital. Pigeons, which have previously been identified as a host of the mites (Deoreo, 1958), were found to be nesting in large sheltered walkways used by the patients. Upon inspection, *D. gallinae* were found in the bird nests (Auger et al., 1979).

A study carried out in four locations in Sweden has shown that there is a genetic difference between *D. gallinae* that infest poultry units and those that infest wild birds. In this study four mite genes were sequenced, three of which were genetically identical. The fourth gene, internal transcribed spacer (ITS) 1, was shown to have two genotypes. The mite populations collected from wild birds had one genotype and the mites collected from poultry units had the other. This genetic difference clearly shows that there are two subspecies of *D. gallinae*. It was thought that the wild bird nests would not serve as a reservoir for the mites from the poultry unit (Brannstrom et al., 2008)

1.3 Poultry housing systems

1.3.1 Battery cage system

Battery cages house laying hens in a relatively small area to allow thousands of hens to be kept efficiently and away from predators in a poultry house. The floor of the cage is sloped allowing the eggs to roll to the front to be collected on a conveyor belt. Figure 1.3 is a photograph of a battery cage system.

In the UK 58 % of laying hens were housed in battery cages in 2008 (British Egg Industry Council). In 1988 the European Union issued a Council Directive that laid

out minimum requirements for conventional battery cages. They were to give laying hens "at least 450 cm^2 of cage area". Feeding and water troughs should always be made available to the hens in a battery cage (European Union Council Directive, 88/166/EEC).

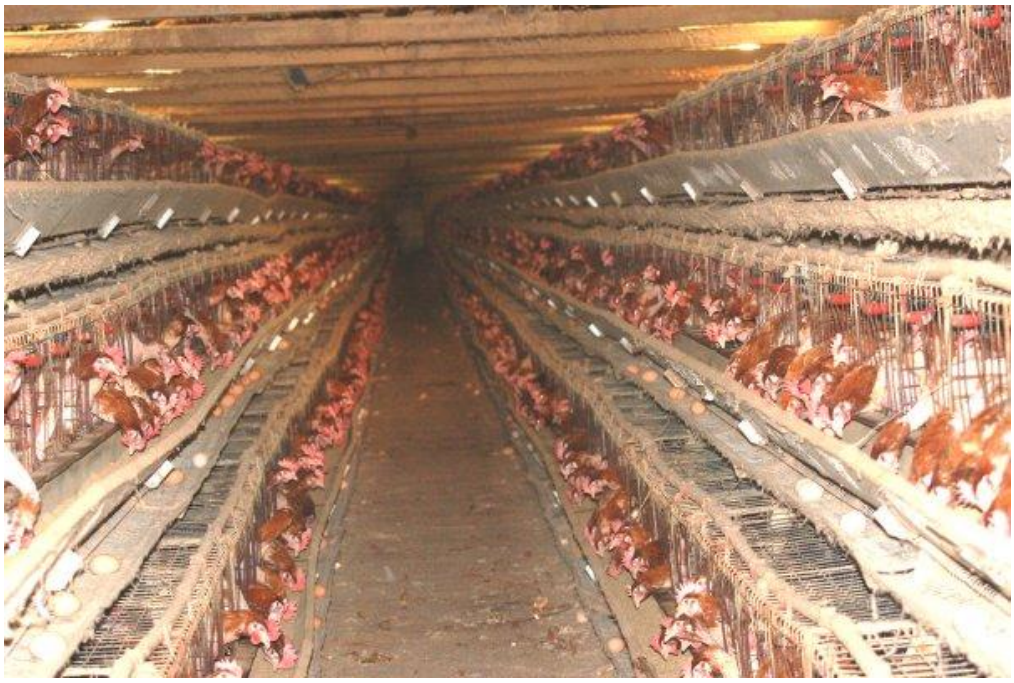


Figure 1.3. Battery cage system containing hens. The hens feed through the bars, which rub their feathers causing bald patches.

This Council Directive was reassessed in 1999 when the distinction between enriched and unenriched caged systems (conventional battery cages) was introduced and the minimum requirements for housing laying hens were increased. The new Council Directive (European Union Council Directive, 88/166/EEC) changed two points of the existing legislation for unenriched cages. Firstly the minimum cage area per hen was increased to 550 cm^2 and secondly the cage had to be fitted with "claw-shortening devices" to prevent overgrown claws. Conventional cages were not allowed to be built from 1 January 2003 and have to be entirely replaced with enriched cages by 1 January 2012. Minimum specifications were also set out for enriched cages in this Directive. Each bird must have at least 750 cm^2 of floor space of which 600 cm^2 should be at least 20 cm high and the cage should be no smaller than 2000 cm^2. Each cage must also have facilities that allow the hens to express

natural behaviour, namely nesting, roosting and pecking or scratching. To this end, a curtained nesting area, a roosting perch allowing at least 15 cm per hens and a litter or scratch pad must all be provided. On the management side the aisles between the rows of cages must be at least 90 cm wide and there must be at least 35 cm between the bottom of the cage and the floor (Council Directive, 1999/74/EC). The implementation required in this legislation, namely the increase in furniture around the cage, had a negative effect of increasing the number of hiding places for *D. gallinae*. This also made cleaning the cages more difficult, and in the long term larger mite populations would be able to hide during the cleaning effort.

The environmental conditions in a poultry unit allow the growth of mite populations. In a battery cage unit there are many dark places away from the hens where the mites can hide. Due to the number of hens in a battery unit the temperature is maintained at about 25°C, which is an optimal temperature for mite population growth.

In battery cage farming systems the lighting regimen is carefully monitored. Hens that have a higher photostimulation show increases in egg production whereas a reduction in length of light stimulation can delay or decrease production. Hens have extra-retinal photoreceptors located in the brain. These receptors are affected by red light that stimulates egg production. Hens exposed to blue or green light stimulation laid fewer larger eggs (Rozenboim et al., 1998). The length of the light period was chosen to mimic the length of days during summer so that the hens will lay eggs all year. The artificial lights used in poultry units are required to be kept dim to reduce the amount of pecking that can lead to injury (Prescott & Wathes, 1999). During a normal legal lighting regime, where there has to be at least eight hours of uninterrupted darkness, *D. gallinae* spends most of it's time off host. However a study was performed that changed this regime to create varying periods of light and darkness. The lighting regimes followed over a 12-week period were: group 1 had 14 hours of light followed by 10 hours of darkness; group 2 had 20 hours of light followed by four hours of darkness; group 3 had four repeats of 3.5 hours of light followed by 2.5 hours of darkness; group 4 had 24 repeats of 15 minutes light followed by 45 minutes darkness. The mean number of eggs per hen, mean feed

intake and number of mites collected were measured. Groups 3 and 4 had the smallest number of mites recovered no mites were collected from group 3 and only a single mite was found in the group on the last week of the study in group 4. These groups also laid the most eggs on average (60 eggs from each group) but on average did not consume the largest mass of feed, 13 kg and 11 kg respectively, compared to 12.5 kg consumed by group 1 and 15 kg consumed by group 2, who laid 59 and 56 eggs respectively. The only group that had a legal light regime was group 1; the hens laid 59 eggs on average and ate 12.5 kg of feed. However the highest number of mites was collected from this group, 158 mites collected in total with 124 of these collected during week 12 (Stafford et al., 2006).

1.3.2 Free range system

Free range housing systems for laying hens differ from the battery cage system in that the hens are not restricted inside the house and can roam around outside the house. The housing is covered in deep litter, which mainly consists of sawdust, and the hens are given nest boxes where they can lay their eggs. To be called free range, the stocking density has to be fewer than 1000 hens per hectare (Farm Animal Welfare Council, Report on the welfare of laying hens, http://www.fawc.org.uk/reports/layhens/lhgretoc.htm).

1.3.3 Barnyard system

The barnyard system is very similar to the free range system except the hens are kept in a large barn but do not have access to a pasture on which to roam. In a survey conducted in England, it was observed that flocks of laying hens housed in barnyard systems were more heavily parasitized than flocks in free range and even battery cage systems (Fiddes et al., 2005).

1.3.4 Organic

The differences between organic and other non-caged egg production system include feed, animal welfare and medication. The hens are not caged, they cannot be fed animal by-products or genetically modified crops and the use of chemicals are limited (Zenner et al., 2003). Farmers are only allowed to treat flocks with antibiotics in cases of emergency.

1.4 Control methods

Control methods at present rely on the use of chemicals, for example Poultry Shield and ByeMite®, while hens are in the cages (described in 1.4.1). However the mites live in hard to reach places that cannot easily be cleaned. There are differing reports on the most heavily infested housing system; one paper suggests that *D. gallinae* can infest free range systems to a higher degree than battery cage systems (Arkle et al., 2006), whilst a survey of poultry farmers in another suggested that barnyard systems were more heavily infested than battery cage systems (Fiddes et al., 2005). Barnyard and free range housing systems have more places for mites to hide, and the hens are kept on the floor in these systems, which make the use of chemical sprays directed specifically at the mites more restrictive. Cages however can be cleaned and sprayed directly using steam jets. However, time is restricted in a commercial poultry unit, and it is not possible to de-infest entire barns before the next flock arrives, due to the losses that are accrued during down time where the hens are not producing eggs.

1.4.1 Chemical control

As previously stated *D. gallinae* lives in small, dark spaces that can be hard to reach, so cleaning of cages by spraying needs to be meticulous in order to be effective. Spraying of cages can be unidirectional, leaving large mite populations on untreated sections of the cage (personal observation). Due to the short-lasting action period of some of the chemicals used, they need to be directly applied to the mites to have any

effect. For example, Poultry Shield is a detergent-based treatment, which can be sprayed into crevices in the poultry cages that the mites hide in during the day. Poultry Shield penetrates the cuticle of the mite and the protective coating around the egg, causing dehydration and death.

Historically, a number of chemical acaricides have been used to control *D. gallinae*, for example synthetic pyrethroid acaricides such as Permethrin, and organophosphates such as dichlorvos. These chemicals have been used successfully for many years to control parasite numbers, although parasites have now started to develop resistance, and their use has been discontinued (Beugnet et al., 1997). On farms which appeared to have problems with chemical resistance, between eight and 40 times as much Permethrin was required to kill 50 % (LD50) of the mites compared with a control farm (Beugnet et al., 1997). In addition, these chemicals may be toxic to host animals, including humans (Nordenfors et al., 2001) and can contaminate the environment and food products destined for human consumption (Mulenga et al., 2000). For example, residue of Propuxur has been found in eggs at a level six times higher than the maximum permitted in the European Union (Hamscher et al., 2003).

Ivomec® (Merck Sharp and Dohme S.A.), which contains ivermectin, of the macrocyclic lactone family of anthelmintics, has been tested to ascertain its possible use in the control of *D. gallinae*. A range of concentrations of Ivomec® were injected intra-abdominally or intra-muscularly into hens. Ivomec® injected at a dose of less than 0.6 mg kg^{-1} intra-abdominally or when injected intra-muscularly at any concentration had no visible effect on the mites, which could still lay viable eggs. When administered intra-abdominally, at a dose of above 1.8 mg kg^{-1}, Ivomec® had a noticeable effect. At a dose of 5.4 mg kg^{-1}, the mites suffered paralyses, which lasted a maximum of 70 hours. However this is close to the dose that is toxic to experimental hens (Zeman, 1987).

The chemical ByeMite®, developed by Bayer HealthCare (Animal Health Division), is an emulsifiable concentrate containing phoxim (50 %), an organophosphate. The

efficacy of the drug was assessed during a trial on two commercial poultry farms where hens were housed in battery cages. ByeMite® was used to treat one farm and the other was untreated. Traps were put out on eight occasions from the day before treatment until 49 days post-treatment. Efficacy, measured in terms of reduction in mite numbers on the treated farm when compared to the control farm, was 96 % on day three and 99.8 % by day 49 (Meyer-Kuhling et al., 2007). *D. gallinae* from two farms in Germany and France were tested *in vitro* with ByeMite® and a biological product Mite-Stop®, which was derived from seeds of the plant *Azadirachta indica*. ByeMite® was shown to be as effective as previously demonstrated, killing 96 % of the mites in the experiment from the French farm. The percentage mortality caused by ByeMite® when the mites were in contact with the drug for only four seconds dropped to 27.8 % with mites from the German farm and 30 % with mites from the French farm. Mite-Stop® caused 100 % mortality on the mites from the French farm and 84.5 % mortality on mites from the German farm (Abdel-Ghaffar et al., 2009). The ineffectiveness of ByeMite® results from survival of mites due to their ability to hide in the crevices around the battery cages during the short exposure time.

1.4.2 Plant extracts

In a study, *D. gallinae* were exposed to a panel of 56 essential oils and the mortality of mites was measured. When the essential oils, at a concentration of 0.35 mg cm^{-2}, were used in a filter paper contact bioassay only two of the oils failed to cause a mortality of at least 89 %. When the concentration was reduced to 0.07 mg cm^{-2}, 37 of the oils caused a mortality of 50 %, 13 of which caused mortality in all the mites tested. Only two of the oils cade (*Juniperus oxycedrus*) and horseradish (*Cocholearia armoracia*) caused 100 % mortality when the concentration was reduced to 0.04 mg cm^{-2}. When the concentration of oils was lowered to 0.02 mg cm^{-2}, the highest percentage mortality of mites was 46 % with Lime dis 5F (*Citrus aurantifolia*) (Kim et al., 2004). Further studies carried out revealed that the oils had a greater effectiveness in closed containers suggesting that it was the vapour phase that had the acaricidal properties (Kim et al., 2007).

Other studies have been carried out to assess the acaricidal effects of essential oil plant extracts. Thyme (*Thymus vulgaris*) and cade were tested over a time course from 0 to 24 hours. At time zero the thyme caused an increase in mortality of about 5 % and increased to 50 % by 2 hours. The cade caused a mortality of 50 % only after 24 hours (George et al., 2010a). Another essential oil used in an *in vitro* trial was manuka (*Leptospermum scoparium*). This caused a mortality of almost 100 % in juvenile mites and almost 90 % in adult mites 24 hours after exposure. However, the ovicidal mortality was less than 10 % (George et al., 2010b).

Plant products that have acaricidal properties could be used to control *D. gallinae* in the short term. However, as discussed in relation to chemical control, resistance in mite populations could develop against plant-derived products as it has done against organophosphates. Plant derived products, which are a possible alternative to chemical control that may not have a withdrawal period, could be used in combination with vaccination to control *D. gallinae*.

1.4.3 Current vaccination strategies

At present there is no vaccine for the control of *D. gallinae*. Hens however are vaccinated against a variety of other diseases, as detailed in Table 1.1.

Immunological control, involving the use of vaccination with protein antigens, would allow farmers to reduce the number of chemical treatments required to bring mite populations to a manageable level. A reduction in use of acaracides is desirable for several reasons; the build up of drug resistance will be slowed, there will be fewer potentially toxic residues in food products, and exposure to potentially harmful chemicals by poultry workers will be reduced. Vaccination will be further discussed in 1.5.4.

1.5 Avian immune system

Studies of the avian immune system have contributed to critical discoveries in the field of immunology, not least of which was the separation of bursa of Fabricius derived (B) lymphocytes and Thymus derived (T) lymphocytes that comprise the adaptive arm of the immune response (Davison, 2003).

The immune system of birds has a number of similarities to that of the mammalian immune system. For example, both systems can be split into an innate and adaptive response.

Table 1.1. Vaccines currently available to poultry farmers.

Vaccine Name	Vaccine type	Disease	Administration	Manufacturer
AviPro SALMONELLA VAC E	Live vaccine	Salmonella enteritidis	Drinking water	Lohmann Animal Health
AiviPro REO	Live vaccine	Viral Arthritis and Tenosynovitis	Intramuscular or subcutaneous injection	Lohmann Animal Health
AiviPro P OX	Live vaccine	Fowl Pox	Intracutaneous injection	Lohmann Animal Health
AiviPro ND VISOTA	Live vaccine	Newcastle Disease	Drinking water, intraocular, intranasal or spray application	Lohmann Animal Health
AiviPro MD BIVAC	Live vaccine	Marek's Disease	Injection	Lohmann Animal Health
PRECISE	Live vaccine	Infectious Bursal Disease	Drinking water	Lohmann Animal Health
IB M48	Live vaccine	Infectious Bronchitis	Drinking water, intraocular, intranasal or spray application	Lohmann Animal Health
Avian Influenza (H1-H5, H7, H9, H10)	Inactivated oil emulsion vaccine	Avian Influenza	Subcutaneous injection	Lohmann Animal Health
AviPro 104 MG Bacterin	Inactivated oil emulsion vaccine	Mycoplasma gallisepticum	Subcutaneous injection	Lohmann Animal Health
AviPro 109 SE4	Inactivated oil emulsion vaccine	Salmonella entertidis	Subcutaneous injection	Lohmann Animal Health

1.5.1 Innate immunity

The avian and mammalian innate immune systems perform the same function and have many of the same processes in common. Mucosal tissues in both systems offer the first line of defence against pathogens. Two of the main mucosal tissues that are under constant pressure from pathogens are the gut and the respiratory tract. There are specific areas in both tissues that allow the interaction between parts of the innate immune system and adaptive immune system. These are the gut-associated lymphoid tissue and the bronchial-associated lymphoid tissue. Antigen presenting cells, such as dendritic cells, migrate to these tissues after they have been activated by pathogens. In the lymphoid tissues, the dendritic cells interact and stimulate T lymphocytes in an antigen specific immune response.

The complement cascade is a series of serum based proteins that are sequentially activated in three ways. 1) The classical pathway is activated by antibody-antigen complexes. In mammals these antibodies are IgG and IgM, however in the avian immune system IgY, the functional equivalent of IgG, does not activate complement (Cova, 2005; Koch et al., 1982). Some of the proteins that are specific to the classical complement pathway have been characterised in hens suggesting that the pathway is present. 2) The lectin pathway is activated by lectin proteins binding pathogen associated molecular pattern (PAMP) receptors. 3) The alternative pathway is activated by natural molecules such as bacterial lipopolysaccharides, zymosan (yeast), fungi, viruses and aggregates of immunoglobulin. Only a single protein has been characterised in hens that forms part of this pathway, factor B (Kjalke et al., 1993), but the presence of this protein would indicate that the pathway is present. The complement cascade opsonises pathogens for phagocytosis and forms a membrane attack complex (MAC) that integrates with cellular pathogen's membrane, allowing cell lysis. The proteins that are involved in both these processes have been identified in hens (Mikrou & Zarkadis, 2010).

1.5.2 Adaptive immunity

The adaptive immune response is activated by parts of the innate immune response, which in turn focuses the innate response, making it more effective. This is the same in both birds and mammals. The adaptive immune response responds faster and with a greater specificity to re-infection by the same pathogen.

The cellular immune response involves the activation of lymphocytes, namely thymus-derived (T) cells and bursa-derived (B) cells. T cells, which discriminate between self and non-self and are responsible for cell-mediated immunity, partially mature in the thymus in both the avian and mammalian immune systems. The maturation of B cells, which produce immunoglobulins, occurs in the bursa of Fabricius in the avian immune system, whereas this occurs in bone marrow in the mammalian immune system. The bursa of Fabricius is a spherical lymphoid organ situated near the hen's cloaca. It has been shown that the removal of the bursa at progressively later stages of development in chicks can preventing antibody class switching by B cells as well as blocking antibody production permanently (Davison, 2003). It has been shown that the bursa is also involved in the immune response to local antigen around the cloaca (Rose, 1979). The bursa can pinocytose antigens across its epithelium like the Peyer's patch does in mammals.

The lymphoid tissue is arranged in a totally different manner in birds than in mammals. There are no organs with the same structure as mammalian lymph nodes in birds. However there are tissues that have two compartments that separate and regulate the interaction of lymphocytes in the same way as mammalian lymph nodes. The precursors of both types of lymphocytes differentiate in the primary lymphoid tissue, which is bone marrow derived for both mammals and birds. B cells in birds differentiate further in the bursa and migrate to the lymphoid tissues whereas with mammals they interact with T cells and mature into plasma cells or memory cells. There are peripheral lymphoid tissue in birds, such as the spleen, which is the same in mammals. In birds there are other tissues that bring the lymphocytes together, such

as mural lymphoid nodes, aggregated nodules of the digestive tract and occlusion region, as well as many smaller solitary nodes scattered throughout the body.

1.5.3 Avian antibody

During this study, experiments were performed involving the injection of protein antigens into hens to generate an immune response. This was subsequently tested to ascertain if the immune response generated would protect hens from *D. gallinae* infestation. These experiments specifically investigated the antibody response that was monitored using Western blot analysis and an *in vitro* feeding assay (McDevitt et al., 2006; Wright et al., 2009).

Antibodies are generated from four protein molecules, two heavy chain molecules and two light chain molecules, which form a Y shaped structure. The molecule has two fragment antigen binding (Fab) regions, which bind specific epitopes on antigens and a single fragment crystallisable (Fc) region. The Fab regions comprise of portions of the heavy and light chains. The Fc region is made from two heavy chains and recruits effector cells and molecules to destroy the pathogen bound to the Fab receptor (Abbas & Lichtman, 2005). The antibody response in mammals and in birds is T cell-dependent but antibodies are produced by B cells. There are three classes or isotypes of antibody that birds produce; IgM, IgY and IgA (Davison, 2003). Systemic IgM is the first class of antibody to be produced in both mammals and birds following antigen challenge. Class switching then occurs during which the mRNA of some the B cells is spliced so that a different antibody heavy chain and therefore a different antibody class, IgY or IgA, is translated and expressed. The light chains are not altered during antibody class switching. The Fab region remains the same after class switching but the Fc region at the end of the heavy chain changes. The other mammalian antibody classes, IgE and IgD, are not present in birds. It has been hypothesised that IgY is an evolutionary ancestor of the mammalian IgG and IgE (Warr et al., 1995). IgY and IgM are systemic antibodies, whereas IgA is a local antibody that can be found predominantly in mucus and secreted fluids (Abbas &

Lichtman, 2005). The only class of antibody in egg yolk is IgY, which can be purified easily from the yolk in large quantities (Schade et al., 1994).

After vaccination with a protein antigen, systemic antibodies are raised that can bind to, and impair the function of, the target protein. After mammalian antibodies bind to their target epitope the classical pathway of the complement cascade can be activated. However, IgY has a different Fc region to mammalian IgG that cannot activate the avian complement cascade (Cova, 2005).

1.5.4 Vaccination

Vaccination is the process by which the immune response is primed against an antigen. The antigen presented to the immune system can be an attenuated parasite, virus or bacterium, or a sub-unit vaccine such as a mix of native proteins or a recombinant protein. The experiments described in this thesis involved immunisation of hens firstly with a mix of native proteins extracted from the mite using the natural solubility and then charge of the proteins to fractionate. During the thesis this process was called the "pragmatic" approach to vaccine development, which was first described during the production of the vaccine TickGARD™ (Willadsen, 2004). A second series of experiments were performed where hens were immunised with recombinant proteins that have implied critical function and had either been employed in immunisation trials against other parasites or were identified in expressed sequence tag (EST) database, which was called the "genetic" or "rational" approach to vaccine development throughout this thesis.

During a natural parasitic infestation the host is continually being exposed to antigens from the parasite. Exposed antigens are proteins that are processed or "seen" by the host's immune system (Willadsen & Kemp, 1988). An immune response generated against exposed antigens is an acquired or "natural" immune response. The acquired immune response may be protective thereby reducing the level of parasitism that the host is subjected to (Trager, 1939). A study was performed using hens and *D. gallinae* where no significant relationship was found between anti-mite IgY

antibody titre and mite population. However there was a significant relationship between the mite population and the mortality of the hens (Arkle et al., 2006). The exposed antigens from *D. gallinae* do not appear to generate an anti-mite immune response to moderate the exponential population growth, which can be observed in poultry houses during the mites' optimum growing conditions. However, naturally acquired immunity has been demonstrated in White leghorn hens against the NFM. The number of NFM infesting a population of hens has been shown to be inversely related to the number of mites that re-infested the same hens after the hens had moulted, which deprived the mites of suitable conditions for infestation, i.e. the hens that had an initially high mite count had fewer mites after re-infestation (DeVaney & Ziprin, 1980). A study conducted with NFM and hens proposed two hypotheses about the decline of NFM populations in hen flocks: 1) The local inflammatory response in the skin blocks the NFMs access to the blood and 2) the Major Histocompatibility Complex affects the intensity of the inflammation response. It was observed that on infested hens the NFM lives on the border of sites of inflammation caused by an immune response, migrating out from the initial point of infestation (Owen et al., 2009). Decreased contact between *D. gallinae* and their host could be a reason why there is little evidence to suggest that hens develop a natural immunity. The NFM lives solely on its host so it is in contact with the hens for longer than *D. gallinae* that only feeds for approximately one and a half hours before withdrawing to the hiding places where it progresses through the lifecycle. The inflammatory response in hens infested with *D. gallinae* would probably be less intense since the mite does not restrict its feeding sites to the border between inflamed and normal skin, as is the case for NFM, so can avoid areas of inflammation on the hen. When confined to a single location the hen could mount an inflammatory response, however a study conducted to measure the mRNA cytokine expression did not indicate that there was a particular immune response generated (Harrington et al., 2010).

Antigens from a parasite that are not presented to the host immune system during a natural infestation, such as proteins from the mite's gut or intracellular proteins, are known as "concealed" antigens (Trimnell et al., 2002). Following injection of

concealed antigens into the host, the antibodies generated in response can bind to these proteins within a feeding parasite and block their normal function, leading to a reduction in population of the parasite (Knox et al., 2003). Vaccination of hens with a concealed protein antigen from *D. gallinae* could elicit a strong protective antibody response that would result in the reduction of the population size of the mites in the poultry house.

The pragmatic approach to vaccine development has previously been explored with *Psoroptes ovis*, an ectoparasite of sheep. *P. ovis* feeds on the exudates produced by lesions caused by the presence of the mite (Van Den Broek & Huntley, 2003). Antibodies present in the exudates have been shown to be ingested by the mite. These antibodies are partially digested by enzymes in the gut (Pettit et al., 2000) but could still be functionally active and be able to bind to protein(s) in the gut of the mite. *P. ovis* mites were sequentially homogenised to produce distinct protein fractions. These protein fractions were then injected into sheep, which were subsequently challenged with live mites. The sheep immunised with the protein fraction that was soluble in a PBS with Tween-20 solution, showed a reduction in lesion size and mite numbers (Smith et al., 2002). Serum from that trial, along with serum from a subsequent trial (Smith & Pettit, 2004), was used to immunoscreen a *P. ovis* cDNA library to identify immunoreactive proteins that could be expressed in a recombinant form. Two proteins of interest were found; a catchin-like protein and a novel mu class glutathione S-transferase. Western blot analysis showed that antibodies were raised against the recombinant forms of these proteins in sheep but were not present in the sheep of the control group, which were injected with adjuvant only (Nisbet et al., 2008). These antibodies could bind to the gut proteins in the mite resulting in an anti-mite effect, which could impair feeding ability that would led to an increase in mortality or a decrease in reproductive capability.

The pragmatic approach was also taken in the development of TickGARD™, which has been the only commercially available vaccine for ectoparasites to date. It is a very effective vaccine against a population of tropical cattle tick *R. microplus*. The vaccine impairs the ability of the tick to digest a bloodmeal, which results a 30%

reduction in the number of engorged ticks, a 30 % reduction in the weight of engorged ticks and an 80% reduction in the weight of eggs laid per gram of engorged female tick, which therefore reduced the number of ticks on pasture. When the measurements listed above were combined a total vaccine capability of 90 % was calculated (Willadsen et al., 1995). The protective antigen in TickGARD™ is Bm86, a glycoprotein attached to the membrane of digest cells that are part of the tick's gut wall. Bm86 is a "concealed" antigen since it is not detected by the host immune system during a tick infestation. Bm86 has an amino acid identity of between 85 % and 94 % between ticks of the same genus (Canales et al., 2008) and an amino acid identity of 34 % between Bm86 and a homologue in *Haemaphysalis longicornis*, a hard tick (Liao et al., 2007). The vaccine generates antibodies in the host against Bm86, which are ingested by *R. microplus* and bind to Bm86 a glycosolated protein. In mammalian blood, immunoglobulins such as IgG activate the complement cascade, resulting in formation of the membrane attack complex. This occurs in the gut of the tick where the complement cascade results in lysis of the digest cells the Bm86 protein is bound to, leading to an inhibition of bloodmeal digestion. This damage results in the reduction in reproduction capacity of ticks feeding on vaccinated cattle (Kemp et al., 1989; Rand et al., 1989; Willadsen et al., 1989).

During the development of TickGARD™, *R. microplus* ticks were homogenised in a Tris buffer to create a crude protein extract. The homogenate was centrifuged, resulting in a water-soluble fraction and a water insoluble fraction. These two fractions were then injected into cattle that were challenged with ticks. The insoluble fraction, which contained the membrane or particle bound proteins, proved the most effective in increasing mortality of the ticks. The detergent Nonidet P-40 was then used to solubilise the membrane bound proteins. The cattle in the group injected with the high molecular weight proteins that were soluble in the detergent had the lowest number of ticks per animal. These high molecular weight proteins were then subjected to isoelectric focusing, and then gel permeation chromatography to further reduce the number of different proteins in each injection (Willadsen et al., 1988). Further purification involving affinity chromatography with a wheat germ lectin-Sepharose column and gel filtration was performed. The protein that was the

effective antigen was named Bm86, a glycoprotein with a molecular weight of 89 kDa (Willadsen et al., 1989). The recombinant form of the protein was used in the vaccines TickGARD™, which was licensed in Australia, and Gavac™, which is licensed in Cuba. Although Bm86-based vaccines are highly effective against *R. microplus* there is a strain of the tick that is resistant, Argentina strain A. This strain of *R. microplus* has a homologue of Bm86 called Bm95. Bm95 was used in an immunisation trial where cattle were challenged with two strains of tick, Argentina strain A and a susceptible strain of *R. microplus* Camcord. A reduction in tick burden was observed on the cattle in both vaccinated groups, the resistant strain was reduced by 58 % and the susceptible strain by 89 % (Garcia-Garcia et al., 2000).

Potentially protective proteins have been used in immunisation trials against endoparasites. Although endoparasites such as the nematode *Haemonchus contortus*, and ectoparasites such as *D. gallinae*, parasitize their respective hosts in a very different way, there could still be similar proteins in both that elicit a protective immune response. *H. contortus* and *D. gallinae* are both parasites that require a blood meal to complete their life cycle. Concealed vaccine candidates that have been identified in endoparasties have the potential to be effective against *D. gallinae*.

Studies using pragmatic fractionation identified a complex structure in the intestine of *H. contortus* to be *Haemonchus* galactose-containing glycoprotein complex (H-gal-GP) (Smith et al., 1994). Recombinant versions of all the proteins that form the H-gal-GP protein complex were injected into sheep, which were subsequently challenged with *H. contortus* larvae. Sheep were also injected with the native form of the complex purified from *H. contortus*. Antibodies were detected that recognised H-gal-GP were detected in both the native and recombinant groups, however only the group injected with the native form of the protein complex developed protective immunity to the nematode (Cachat et al., 2010).

By comparison to the pragmatic approaches detailed above, the rational approach to vaccine development employs proteins that have either already been shown to elicit an immune response that causes an increase in mortality in a closely related species

or proteins that are perceived to perform a critical physiological function. It is hoped that *D. gallinae* will show a close protein homology to other species of mites and, especially, ticks. In the field of tick biology, immunisation studies have been performed that test the capacity of many protein antigens to generate a protective immune response. The tropical cattle tick *R. microplus*, which is the only ectoparasite for which a vaccine has been commercially developed, and *D. gallinae* are members of the same Superorder (Parasitiformes). Mite homologues of protective protein antigens discovered in ticks could be used to generate an immune response against *D. gallinae*.

Gavac™ has been used in an immunisation trial using hens where antibodies raised were fed to *D. gallinae*. IgY purified from the yolk taken from eggs laid by the test group (injected with Gavac™), and from eggs laid by the control group (injected with the Montanide ISA 50V adjuvant only), were fed to *D. gallinae* in hen blood using an *in vitro* feeding assay. The mortality of the mites fed antibodies raised against Bm86 was not significantly different from the mortality of the mites fed antibodies from the control hens. This trial also measured the mortality of mites fed antibodies raised against a recombinant form of subolesin, a potential vaccine candidate identified in ticks (Galindo et al., 2009). There was a significant increase in the mortality of the mites fed antibodies raised against subolesin when compared to the control group. Western blot analysis confirmed that antibodies were generated against Bm86 but not against subolesin at the predicted molecular mass. However, there were bands representing higher molecular mass proteins that could have been subolesin multimers. Polymerase chain reaction using gene-specific primers for subolesin and Bm86 amplified two DNA fragments in the subolesin reaction but no products were amplified in the Bm86 reaction. The smaller of the two DNA fragments (150 base pairs (bp)) amplified using the subolesin primers aligned with murine akirin1, a nuclear factor involved in the innate immune response, with a score of 49 %. The larger of the fragments amplified (300 bp) aligned with subolesin in *Amblyomma hebraeum*, with a score of 31 % (Harrington et al., 2009a). The low homology and lack of antibody response against subolesin described above, and the inability to successfully clone either protein at Moredun Research Institute

(experiments conducted by Dr. K. Bartley and author (unpublished data)) suggests that these proteins are not present in *D. gallinae*.

Although there are only a few vaccines that have recombinant proteins as the antigen, such as TickGARD™ and Gavac™, the method has been shown to be a viable alternative to the use of chemicals for the control of pathogenic organisms. Although there are only two vaccines commercially available against ticks, and they use the same recombinant antigen (Bm86), many proteins in a recombinant form have been studied to assess their capacity to generate a protective immune response. The salivary protein 64P from the tick *R. appendiculatus* has been identified as a potential vaccine candidate. 64P is a component of the tick's cement cone that is injected into the host to aid feeding by securing its mouthparts to the skin and is therefore an exposed antigen. Recombinant truncated versions of this protein were injected into guinea pigs, which were then challenged with ticks. Of the six truncated versions of the protein, three caused mortality of between 55.5 % and 70 % in ticks that were used to challenge guinea pigs (Trimnell et al., 2002).

Histamine release factor (HRF) is a protein that has been identified in ticks that is expressed in a variety of their tissues. HRF was also identified in *D. gallinae* and a recombinant form of the protein was used to immunise hens. The antibodies generated were tested in the *in vitro* feeding assay, which resulted in a modest increase in mite mortality to 9.2 % in the test group from a percentage mortality of 2.7 % when mites were fed control blood. Although the function of HRF in *D. gallinae* is unclear it could be employed in a vaccine as part of a cocktail of recombinant proteins since it seems to have an anti-mite effect (Bartley et al., 2009).

At the start of this project the only non-ribosomal protein identified in *D. gallinae* and published on the National Centre for Biotechnological Information database was tropomyosin. Tropomyosin is structural component of the cell cytoskeleton, which was successfully cloned from *D. gallinae* using degenerate primers designed using sequences from ticks and other mite species (Nisbet et al., 2006a). It has been used in immunisation trials against the filarial nematode *Acanthocheilonema viteae*. Both a

recombinant and the native form of *A. viteae* tropomyosin have been used to immunise jirds, which are rodent-like mammals. After challenge the recombinant form of the protein proved as effective as the worm-derived native protein in reducing worm burden by 30 %. Therefore the immune response generated by the host was not derived from any post-translational modifications in the native form of the protein (Hartmann et al., 2006).

Paramyosin is another protein of the cell cytoskeleton. It is a thick filament protein with a coiled-coil structure and is found only in invertebrates. Paramyosin has been demonstrated to elicit an immune response against *Schistosoma mansoni* (Zhang et al., 2006). Paramyosin has also been demonstrated to induce a protective response to *S. japonicum* in mice. Immunisation with 5 µg of native paramyosin extracted from *S. japonicum* reduced the worm burden of experimentally infected mice by up to 86 % after a five week period when the mean number of worms in the control mice and vaccinated mice were counted. By use of Western blot the antibody response generated was shown to be very specific to the antigen (Ramirez et al., 1996).

1.6 Aims and objectives

The aim of this project was to identify potential protein antigens for use as a vaccine to protect against *D. gallinae* infestation in poultry.

The first objective, employing the "pragmatic" approach to vaccine development, was to divide the proteome of *D. gallinae* into protein fractions and inject them into hens to generate antibodies. These antibodies would be tested in an *in vitro* feeding assay for their ability to generate a protective response. The most promising protein fraction would then be subdivided, injected into hens and re-tested in the feeding assay. This process would reduce the number of potential protein antigens that might be effective in increasing mite mortality with a view to identifying the most important ones.

The second objective, employing the "rational" approach, was to sequence genes from a complete cDNA library and a subtractive cDNA library and create expressed sequence tag (EST) databases. Roche 454 sequencing technology would be employed to generate a large EST database from the complete cDNA library. This large database would then be used to identify small sequences generated by Solexa sequencing of mRNA from fed and starved mites. The Solexa sequencing technology would be used to compare the expression levels of genes in two populations of mites. Sanger sequencing would be utilised to generate a smaller EST database using the subtractive library to look for homologues of known vaccine candidates in *D. gallinae*.

The third objective was to sequence the paramyosin gene from *D. gallinae* and use it together with the tropomyosin gene (Nisbet et al., 2006a) to produce recombinant proteins. Hens would then be injected with the recombinant proteins to generate antibodies, which would be tested in the feeding assay and by Western blotting.

The fourth objective was to carry out an *in vivo* trial. The most promising protein fractions identified in objective one and a cocktail of five recombinant proteins, including paramyosin and tropomyosin, identified in objective three, would be used to immunise laying hens, which would then be challenged with live mites. The mite population infesting the hens and the quantities of antibody generated against these proteins would be monitored throughout the experiment.

Chapter 2:

Materials and Methods

2.1 Materials

The materials described in this chapter were generic. Specific materials for individual chapters are described in the materials and methods section of the relevant chapter

2.1.1 *Escherichia coli* strains

The *E. coli* strains used in the experiments were JM109 competent cells (Promega) and BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene).

2.1.2 Cloning Vectors

The cloning vectors used in the experiments are listed in table 2.1.

Table 2.1. Vectors used in this study.

Vector	Size (bp)	Reference
pGEM®-T	3015	Promega
pET-22b(+)	5493	Novagen
pET-SUMO	5643	Invitrogen

2.1.3 Primers

The stock concentration of all the primers was 100 pmol/µl. Primers were diluted to 10 pmol/µl for use in PCR reactions. Table 2.2 lists the standard primers used in the experiments, with their sequences and their Tm.

Table 2.2. Primers used in this study.

Name	Sequence	Tm (°C)
pET-SUMO forward	5' AGATTCTTGTACGACGGTATTAG 3'	57
T7 reverse	5' TAGTTATTGCTCAGCGGTGG 3'	58
M13 reverse primer	5' AGCGGATAACAATTTCACACAGGA 3'	48
M13 forward primer	5' CGCCAGGGTTTTCCCAGTCACGAC 3'	47
Universal primer mix	5' AAGCAGTGGTATCAACGCAGAGT 3'	61

2.1.4 Growth Media

The composition of bacterial growth media is listed in table 2.3.

Table 2.3. Growth media used in this study.

Name	Composition
Luria Bertani (LB) medium	1% Bacto-tryptone, 0.5% Bacto-yeast, 1% NaCl
LB agar	LB medium plus 1.5% Agar
LBG medium	LB medium plus 8 % glycerol

2.1.5 Antibodies

The secondary antibody used for Western blots was a rabbit anti-chicken IgY/HRP conjugate (Sigma). The secondary antibody used for immunolocalisation was a rabbit polyclonal anti-mouse IgG/HRP conjugate (DakoCytomation). The generation of IgY against *D. gallinae* proteins for *in vitro* analysis in the feeding assay is described in chapters 3, 5 and 6.

2.1.6 Buffers

The buffers used during this study are listed in table 2.4.

Table 2.4. Buffers used in this study.

Buffer	Ingredients
TAE	40 mM Tris/acetate, 1 mM EDTA pH 7.2
TE8.5	50 mM Tris pH 8.5, 2 mM EDTA
Tris Buffer Saline	0.8 % NaCl, 0.02 % KCl, 0.3 % Tris, pH 7.4
TBS Tween 20	As TBS plus 0.1 % Tween 20
MES buffer	950 ml H_2O, 50 ml 20x NuPAGE MES
Transfer buffer	50 ml 20x NuPAGE Transfer Buffer, 100 ml methanol, 850 ml dH_2O
Wash Buffer	PBS + 0.5 M NaCl, 0.5 % Tween 80
Binding Buffer without urea	20 mM $NaPO_4$, 0.5M NaCl, 20 mM Imidazole
Binding Buffer with urea	20 mM $NaPO_4$, 0.5M NaCl, 8M Urea, 20 mM Imidazole
Elution Buffer 1	20 mM $NaPO_4$, 0.5M NaCl, 8M Urea, 50 mM Imidazole
Elution Buffer 2	20 mM $NaPO_4$, 0.5M NaCl, 8M Urea, 200 mM Imidazole
Elution Buffer 3	20 mM $NaPO_4$, 0.5M NaCl, 8M Urea, 350 mM Imidazole
Elution Buffer 4	20 mM $NaPO_4$, 0.5M NaCl, 8M Urea, 500 mM Imidazole
Mono-Q column Buffer A	10 mM Tris, 0.01% (w/v) Sodium Azide pH 7.4
Mono-Q column Buffer B	10 mM Tris, 1.0M NaCl, 0.01% (w/v) Sodium Azide pH 7.4

2.1.7 Enzymes

The restriction enzymes used during this study were *Not*1 and *BamH*1 (Promega). The DNA modification enzymes were shrimp alkaline phosphatase and T4 ligase (Promega).

2.1.8 Molecular weight markers

The marker set used in these experiments for protein gels was SeeBlue plus 2 molecular mass markers (Invitrogen).

The marker used for DNA gels was the Ready-Load™ 1 kb DNA ladder (Invitrogen).

2.1.9 Antibiotics

The antibiotics used in this study are listed in table 2.5.

Table 2.5. Antibiotics used in bacterial cultures to select for specific bacteria or plasmids. N/A indicates that the bacterium or plasmid does not have the gene to offer resistance.

Antibiotic	**Plasmid**	**Bacterium**
Ampicillin	pET-22b(+)	N/A
Chloramphenicol	N/A	BL21-CodonPlus® (DE)-RIL
Kanamyosin	pET-SUMO	N/A

2.2 Methods

The methods described in this section were generic. Methods specific for individual chapters are described in the materials and methods section of the relevant chapter.

2.2.1 Determination of protein concentration using the Pierce BCA Protein Assay

The concentrations of the bovine serum albumin (BSA) standards were 2 mg/ml, 1.5 mg/ml, 1 mg/ml, 0.75 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.025 mg/ml. The standards were dissolved in the same diluent as the samples. Each standard (25 µl) was pipetted into each of three wells of a 96 well polycarbonate microtitre plate (Stratagene). The samples were tested undiluted, at 1:10 or 1:20 dilutions in the assay and 25 µl were added to each well in triplicate.

Protein assay reagent A and Protein assay reagent B, form the Pierce kit were mixed in a ratio of 50:1. The detection mixture (200 µl) was added to each well. The plate was incubated in the dark for 30 min at 37°C. Optical densities were read at 562 nm and the program KC4 was used to calculate the protein concentration.

2.2.2 Protein concentration estimation of the red mite urea-soluble fraction

The 2-D Quant Kit (Amersham Biosciences) was used to quantify the concentration of the urea-soluble fraction. To six 1.5 ml tubes 0 µl, 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of 2 mg/ml BSA were added. Approximately 0.5–50 µg of protein sample were added to 1.5 ml tubes. The samples were tested undiluted and at a 1:5 dilution. The samples were run in duplicate.

To each tube, 500 µl of precipitant were added. The tubes were vortexed and incubated for 2 min at room temperature (RT). To each tube, 500 µl of co-precipitant were added, the tubes were mixed by inversion and centrifuged for 5 min at

10,000 x *g*, and the supernatant was removed. The tubes were centrifuged briefly and the excess liquid was removed by pipette. Copper solution (100 µl) and dH_2O (400 µl) were then added to each tube and mixed by vortexing. Working colour reagent was prepared by adding 100 parts of colour reagent A to 1 part of colour reagent B, and 1 ml was added to each tube. Tubes were mixed by inversion and incubated at RT for 20 min. The absorbance was then measured at 480 nm in a spectrophotometer. The absorbances of the standards were used to generate a standard curve from which the concentration of the sample was calculated.

2.2.3 Polyacrylamide gel electrophoresis (PAGE)

PAGE was used to separate proteins of different masses. To prepare a 13 µl sample for denaturation then electrophoresis, 5 µl of 4x NuPAGE® LDS Sample Buffer and 2 µl of 10x NuPAGE® Reducing Agent (Invitrogen-NuPAGE®) were added to the sample. This was vortexed and then centrifuged at 10,000 x *g* briefly to collect the entire sample at the bottom of the tube. The tubes were incubated at 70°C in a waterbath for 10 min. 1x NuPAGE® SDS MES Running Buffer was prepared by adding 50 ml of 20x NuPAGE® MES Running Buffer to 950 ml of dH_2O.

A precast NuPAGE® Bis-Tris gel (Invitrogen) was opened and the wells rinsed with dH_2O. The Xcell *SureLock* Mini-Cell was assembled. The Lower Buffer Chamber was filled with 600 ml of NuPAGE® SDS MES running buffer and the Upper Buffer Chamber was filled with 200 ml of NuPAGE® SDS MES running buffer containing 500 µl of NuPAGE® Antioxidant.

SeeBlue Plus2 markers (7 µl) were loaded into the first lane of the gel and 20 µl of the samples were loaded into the other lanes. The gel was run for 35 min at a constant 200 V.

The gel was removed from its cassette and placed in a large weigh boat containing dH_2O, and incubated at RT on an orbital rocker for 5 min. The dH_2O was changed three times. SimplyBlue stain was added and the gel was incubated at RT on an

orbital rocker for 1 hr. The stain was then discarded and the gel was washed in dH_2O, changing the water every 20 min for 1-2 h depending on how clear the background was. The gel was then photographed under white light.

2.2.4 Antibody (IgY) extraction from egg yolks

IgY was extracted from egg yolk using a modified method based on the IgY Eggstract kit (Promega). Eggs were collected six weeks after the initial injection and the yolks were separated from the egg white. The yolk was carefully rolled onto several layers of paper towel, to remove adhering egg white from the intact yolk sac. The egg yolk was then positioned near the edge of the paper towel and punctured to drain into a universal. Yolk (10 ml) was pipetted into a small Duran bottle. Three volumes of Precipitation Solution A were added at RT and the mixture was stirred for 5 min to precipitate the lipids, then centrifuged at 5311 x *g* for 25 min at 4°C. The supernatant was filtered through Whatman grade 1 chromatography paper to remove particulate matter. The volume of the filtrate was measured and transfered to a clean Duran bottle. A third of the filtrate volume of Precipitation Solution B was slowly added, with stirring, for 5 min to precipitate the IgY. The IgY was pelleted by centrifugation at 5311 x *g* for 25 min at 4°C. To resuspend, the pellet was incubated for 4 hrs on a rocker at RT in half the starting yolk volume of PBS. Working solutions of purified IgY were stored at -20°C.

The concentration of IgY was determined using the nanodrop with an absorbance of 280nm, using 1.33 as the extinction coefficient (i.e., A280/1.33 = concentration in mg/ml).

2.2.5 Western Blots

Western blots were prepared using the Invitrogen iBlot™ system to transfer proteins onto nitrocellulose membranes following the manufacturer's instructions. Briefly, a PAGE gel was prepared as described in 2.2.3. Instead of staining the gel it was washed twice for 5 min in dH_2O.

The iBlot™ was assembled by placing the anode stack on the blotting surface. The gel was then placed onto the nitrocellulose membrane, which was at the top of the anode stack, and the blotting roller was used to remove any air bubbles from between the gel and the nitrocellulose membrane. A piece of filter paper pre-soaked in dH_2O was placed on the gel and again any air bubbles were removed. The cathode stack was placed onto the filter paper and air bubbles were removed. The disposable sponge was placed in the lid of the iBlot™ with the metal contact at the top right corner.

The transfer programme ran for 7 min. At the end of the run, the nitrocellulose membrane was removed and placed into dH_2O. The nitrocellulose was incubated for 2 hrs in Wash Buffer to block the membrane. The nitrocellulose was then incubated in the primary antibody overnight at 4°C on an orbital rocker.

The primary antibody was decanted off and Wash Buffer was used to wash the nitrocellulose six times, changing the buffer every 20 min.

The nitrocellulose was then incubated with the secondary antibody at RT for 2 hrs before it was decanted off and Wash Buffer was used to wash the nitrocellulose six times, changing the buffer every 20 min.

DAB (Sigma) was used to visualise the bands present. One of each of the tablets was dissolved in 5 ml of dH_2O. The nitrocellulose was incubated in the DAB until bands were detected and then nitrocellulose was rinsed with tap water to stop the reaction.

2.2.6 *In vitro* Feeding Assay

This method was based on a protocol described previously (McDevitt et al., 2006) and was modified as follows: The bases of 5 ml cryovial tubes (Nalgene) were cut off and smoothed down. Plastic pastettes, cut at the half way point of the bulb and approximately 2 cm up the stalk, were inverted and used as a blood reservoir and to

hold the chick skin in place. Figure 2.1 is a diagram of the apparatus used. Thoroughly washed skins from one-day-old chicks, that had been plucked and harvested previously and stored in 100 % ethanol at 4°C for two weeks, were cut into 2 cm^2 pieces and placed over the end of the tube with the external surface of the skin facing the mites. The skin was held in place by the blood reservoir. The filter paper, cut into strips 40 mm long and 5 mm wide, was used to gather mites and to place them in the prepared chamber. The screw cap of the chamber was then attached.

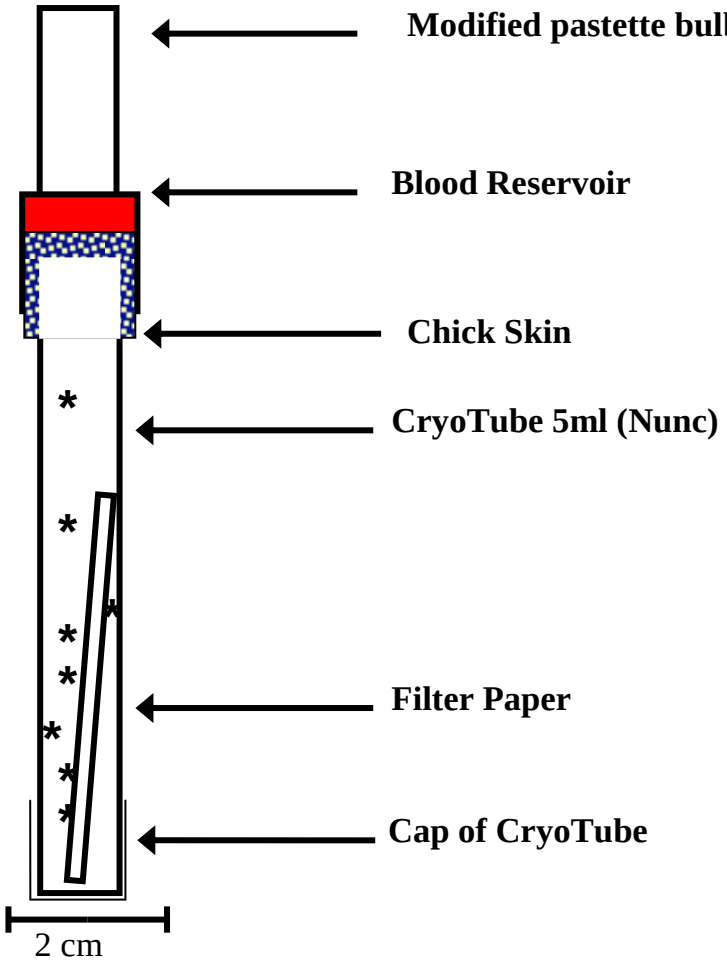


Figure 2.1. *In vitro* feeding device for *D. gallinae*. Method modified from McDevitt et al. (2006).

Extracted antibody was added to fresh heparinised chicken blood (36 USP-units heparin sodium salt /ml blood) and mixed by inversion. The ratios of antibody to blood are detailed in the relevant results chapters. Approximately 300 µl of blood was then added to the blood reservoir and the chambers were incubated overnight at

40°C in darkness, at a relative humidity of 75 % for between 18 and 24 hr. Ten feeding chambers were assembled per treatment group, where the group was either testing antibody from hens vaccinated with mite protein extract or antibody from the adjuvant control group. The experiment was repeated three times for each antibody tested. If the mites had fed within the past 24 hr, i.e. had signs of fresh blood in their gut, they were placed into individual wells of a 96 well plate (Greiner bio-one). Figure 2.2 is a picture of two mites. Panel A has had a recent bloodmeal and panel B has been starved for four weeks.

Figure 2.2. Two pictures of *D. gallinae*. Panel A has recently fed and panel B has been starved for four weeks. The bar on both panels is approximately 0.1 mm.

2.2.7 Immunolocalisation

Fresh, live mites were collected and placed into a 1.5 ml tube, which was filled with Carnoy's fixative. The mites were incubated for 2 hr at RT. The fixative was decanted and 70 % ethanol was added then incubated for 1 hr at RT. The ethanol was removed and replaced with cooled molten 1 % agarose mixed by inversion, then centrifuged at 14,200 x *g* briefly to pellet the mites. After the agarose had cooled, the 1.5 ml tube was filled with 70 % ethanol. This was incubated at 4°C for 1 hr. The

agarose plug was taken from the 1.5 ml tube and any excess agarose was trimmed off. Mite pellets were immersed in molten wax and a cassette was placed on top. Molten wax was poured into the cassette to hold the wax block firm. A microtome was used to cut the block into 5 µm sections. Sections were placed in warm water, to flatten and to allow easy mounting onto glass slides. The slides were then incubated at 40°C for 1 hr to dry. Once dry, the slides were de-waxed, using the following protocol; submerge in xylene of 5 min, submerge in fresh xylene for a further 5 min, submerge in 100 % ethanol for 5 min, submerge in 95 % ethanol for 5 min, submerge in 75 % ethanol for 5 min and then submerge in water for 5 min. After each stage, the excess solvent was shaken off thoroughly. The slides were then washed twice in TBS for 5 min. The natural peroxidases were denatured using 100 µl of 100 % methanol and 1 % hydrogen peroxidase. Normal goat serum (NGS) (100 µl) was used to block the slide, diluted 1:4 in TBS. The slides were incubated for 30 min at RT.

Primary antibody (100 µl) (see results chapters for dilution details) was incubated on the slides overnight at 4°C. The slides were washed twice in TBS, for 5 min each time. The secondary antibody used was 100µl of rabbit anti-mouse Ig/HRP (DakoCytomation) at a 1:200 dilution, incubated at RT for 1 hr. The slides were washed twice in TBS, for 5 min each time, to remove excess secondary antibody. DAB solution was prepared in 5 ml of water and 100 µl was added to each slide and incubated for 10 min at RT. The slides were washed twice in TBS, then twice in dH_2O, for 5 min each time. The slides were immersed in haematoxylin for 30 sec, rinsed with dH_2O, immersed in Scottish tap water for 1 min, then rinsed in dH_2O. The following protocol was used to dehydrate the slides; immerse in 75 % ethanol at RT for 5 min, immerse in 95 % ethanol at RT for 5 min, immerse in 100 % ethanol at RT for 5 min, immerse in xylene at RT for 5 min and finally immerse in xylene at RT for 5 min. The slides were kept in xylene to prevent them from drying out. DPX mountant (Sigma Aldrich) was then used to mount coverslips over the mite sections.

2.2.8 Polymerase Chain Reaction (PCR)

Each PCR reaction was set up using 40 µl of water, 1 µl of 1 µmol forward primer, 1 µl of 1 µmol reverse primer, 1 µl of 50x dNTPs, to a final concentration of 0.25 mmol per dNTP, 5 µl of 10x buffer, 1 µl of template DNA and 1 µl of polymerase, with 5 *u* of activity, in a 0.5 ml PCR tube. The primers used are listed in the relevant results chapters.

The programme run was determined by the annealing temperature (Tm), supplied by Eurofins MWG Operon, of the primers and the length of DNA to be amplified (extension time). A typical programme used was 94°C for 5 min, then 25 cycles of 94°C for 30 sec, Tm-5°C for 30 sec and 72°C for extension time, and finally 72°C for 7 min. The samples were stored at 4°C until required. The Tm and extension times can be found in the Materials and Methods sections of results chapters

2.2.9 Agarose Gel Electrophoresis

GelRed solution (3x) (Biotium, Hayward, USA) (20 ml) and 6 ml of 10x TAE were added to 34 ml of dH_2O in a 250 ml Duran bottle. A 1 % agarose gel was prepared by adding 0.6 g of agarose. The agarose suspension was melted in a microwave oven on full power for 1 min. The bottle was placed in a 50°C water bath to cool before it was poured into the electrophoresis tray.

Once the gel had set, the tray was placed into a tank and filled with 1x TAE until the gel was fully covered. The gel was then loaded, leaving one lane for a DNA ladder. The sample (5 µl) was added to 1 µl of 6x DNA sample buffer. The gel was run using a constant voltage of 80 V for between 40 min and 1.5 hr. The gel was photographed under UV light.

2.2.10 Transformation of E. coli

A screw top polythene tube was placed on ice and left to chill for 5 min. Competent cells were thawed on ice. Plasmid or ligation reaction (2 µl) (= ca. 250 ng) and 50 µl of JM109 competent cells (Stratagene) or BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene), stored at -80°C, were pipetted into the bottom of the chilled test tube. The tubes were left on ice for 30 min and then immersed in a 42°C water-bath for 50 sec to allow the cells to take up the plasmid. The tubes were placed on ice for 2 min. The volume was made up to 1 ml using LBroth and incubated at 37°C with shaking, at 250 rpm for 1.5 hr. Cell suspensions (20 µl and 200 µl) were plated out onto agar plates. The plates were incubated at 37°C overnight.

2.2.11 Plasmid purification using Wizard® *Plus* SV Minipreps DNA Purification System (Promega)

The Wizard Plus SV Miniprep DNA purification system (Promega) was used to purify plasmids from bacterial cells.

An overnight 10 ml liquid culture of transformed bacteria was centrifuged at 1912 x *g* for 10 min. The supernatant was then decanted and the tubes left to stand upside down for 1 min on a paper towel to remove all excess fluid. Cell Re-suspension Solution (250 µl) was added to re-suspend the bacteria and to transfer the suspension to a 1.5 ml tube. Cell Lysis Solution (250 µl) was added and the suspension cleared quickly as the cells lysed. The 1.5 ml tube was inverted four times and then left to incubate for 5 min. Alkaline Protease Solution (10 µl) was added and left to incubate for 5 min to degrade proteins. Neutralisation Solution (350 µl) was added to stop the reaction, and the tube was inverted several times to mix the contents. The 1.5 ml tube was centrifuged at 14,200 x *g* for 5 min and the supernatant was retained.

The supernatant was pipetted onto a Minicolumn. The Minicolumn was centrifuged at 14,200 x *g* for 1 min to allow the DNA to absorb to the column and the eluate was

discarded. Column Wash Solution (750 µl) was added to the Minicolumn, which was centrifuged for 1 min. The eluate was discarded. Column Wash Solution (250 µl) was added. The Minicolumn was centrifuged for 2 min and the eluate was again discarded before the minicolumn was centrifuged again at 14,200 x *g* for 1 min to dry the filter. The Minicolumn was placed in a fresh 1.5 ml tube and 50 µl of dH_2O were added to the centre of the column to elute the DNA. The tube was centrifuged at 14,200 x *g* for 2 min. The plasmid DNA was stored at -20°C.

2.2.12 Pouring agar plates

LB agar (200 ml) was melted at 110°C and cooled to 50°C in a waterbath. Antibiotics were added and mixed into the molten agar, which was then poured into petri dishes. Plates were stored at 4°C until required.

2.2.13 QIAquick PCR Purification kit (QIAGEN®)

Five volumes of Buffer PB were added to one volume of PCR product. A QIAquick spin column was placed in a 2 ml collection tube. To bind DNA, the sample was decanted into the QIAquick column and centrifuged at 14,200 x *g* for 1 min. The flow-through was discarded and the column was replaced in the collection tube. The column was washed by adding 0.75 ml of Buffer PE to the column, which was centrifuged again at 14,200 x *g*. The flow-through was again discarded and the column was replaced in the collection tube and centrifuged at 14,200 x *g*. The column was then placed into a clean 1.5 ml tube. To elute the DNA, 30 µl of water were added carefully onto the centre of the column, which was then centrifuged for 1 min at 14,200 x *g*. The purified PCR product was stored at -20°C until required.

2.2.14 PCR clean up using Exonuclease 1 and Shrimp Alkaline Phosphatase (SAP)

A Master Mix was prepared using of 135 µl of SAP dilution buffer, 100 µl of SAP and 15 µl of Exo 1 per plate. To each well, 2.5 µl of Master Mix was added. The

plates were run on a PCR machine using the following programme: 37°C for 40 min followed by 80°C for 15 min.

2.2.15 QIAquick gel extraction kit (QIAGEN®)

The DNA band was cut from the gel, placed in a clean 1.5 ml tube and weighed. Three volumes of Buffer QG were added per one volume of gel. The tube was incubated at 50°C for 10 min, vortexing every 2 min to dissolve the gel slice. One volume of isopropanol was added and the tube was inverted to mix. The sample was applied to a QIAquick spin column, centrifuged for 1 min at 14,200 x *g* and the flow-through was discarded. Buffer QG (0.5 ml) was added to the column, which was then centrifuged at 14,200 x *g* for 1 min and the flow-through was discarded. Buffer PE (0.75 ml) was added to the column to wash, and the column was centrifuged at 14,200 x *g*, discarding the flow-through. The QIAquick column was placed into a new 1.5 ml tube and 50 µl of dH_2O was added to elute the DNA. The column was centrifuged for 1 min at 14,200 x *g*, and the sample was stored at -20°C until required.

2.2.16 Sanger sequencing

DNA samples were diluted to 8 ng/µl. DNA (5 µl) was added to 5 µl of 3.2 pmol/µl of primer in a 0.5 ml tube. Primers used are listed in the relevant results chapters.

Samples were sent to the Eurofins MWG Operon for DNA sequencing.

2.2.17 Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-ToF)

Protein bands were cut from SDS-PAGE gels under sterile conditions and placed in sterile 1.5 ml tubes. Gel pieces were washed three times in 400 µl 50 % acetonitrile in 25 mM ammonium bicarbonate (pH 8.0) for 15 min. The gel pieces were washed in 100 % acetonitrile for 5 min before being dried in a speed-Vac for 30 min.

The gel pieces were covered with trypsin solution (10 µg/ml trypsin (Promega) in 25 mM ammonium bicarbonate [pH 8.0]) and allowed to rehydrate for 10 min. After 10 min, if all the solution was absorbed, a further 5 µl of trypsin were added. The samples were then incubated overnight at 37ºC. After incubation, 10 µl of 50 % acetonitrile (Rathburn, 5 % trifluroacetic acid (TFA) [Fluka]) were added and the sample was vortexed for 30 min.

For MALDI analysis, 0.5 µl of supernatant was mixed with 0.5 µl of matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in 50 % acetonitrile, 0.1 % TFA) and spotted onto the sample well of a MALDI sample plate. Pepmix 2 standards (1µl [Bruker Daltonics]) were mixed with 9 µl matrix solution, and 0.4 µl was spotted adjacent to each sample spot. Spots were allowed to dry completely before being analysed using the Ultraflex II MALDI-ToF-ToF mass spectrometer. Data for Peptide Mass Fingerprint (PMF) analysis was accumulated from 10x100 shot batches. Each sample was calibrated using the adjacent standard. Where required, further analysis was carried out by obtaining ion fragmentation on individual peptides (Mass Spectrometer/ Mass Spectrometer [MS/MS]).

Data was processed using Flex analysis software and compiled into mass lists. Searches were performed using Mascot^{TM} software, searching the Uniprot and NCBI non-redundant protein databases. Mass tolerances were set at 50 ppm for PMF and 0.5 Da for MS/MS data.

2.2.18 Concentration of protein by centrifugation column (Ambion)

Dilute protein was added to the upper chamber of the centrifugation column. The apparatus was centrifuged at 3000 x *g* for 5 min when the volume was reduced to 750 µl. The concentrated protein was then transferred to a 1.5 ml tube.

2.2.19 Ligation of DNA into pET-22b(+), pGEM®-T or pET-SUMO plasmid

A ligation reaction was set up with 75 ng of PCR product to be ligated with 25 ng of plasmid. T4 ligase (Promega) 2 *u* (1 µl) was added to 15 µl of PCR product, 4 µl of plasmid and 2 µl of 10x ligation buffer. The ligation was incubated for 3 hr at RT.

Ligation into pGEM®-T vector (Promega) uses the A to T overhang. Approximately 3 µl of DNA was ligated into 1 µl of vector (50 ng), 5 µl of 2x ligation buffer and 1 µl of T4 ligase (2 Weiss units/µl) was added to a 1.5 ml tube. The ligation was incubated overnight at 4°C.

The ligation of DNA into pET-SUMO vector was by A to T overhang. Approximately 150 ng of DNA (1 µl) was ligated into 50 ng (2 µl) of vector using 2 *u* of T4 ligase (1 µl), 1µl of 10x buffer and 5 µl of water. The ligation was incubated at 15°C overnight. Ligations were stored at -20°C until required.

2.2.20 Glycerol stock

To store bacteria at -80°C, 0.5 ml of a bacterial culture was added to 0.5 ml of glycerol in a 1.5 ml tube and mixed by vortex.

2.2.21 Silver stain of PAGE gels

The SilverQuest™ kit (Invitrogen) was used to stain PAGE gels prepared with low quantities of protein.

The kit was used as per manufacturer's instructions. Briefly, each gel was washed with 100 ml of dH_2O for 10 ml on a rocker. The gel was then incubated for 20 min on a rocker in 100 ml of Fix (ethanol 40 ml, acetic acid 10 ml, dH_2O 50 ml). The gel was washed in 100 ml of 30 % ethanol for 10 min, then incubated in 100 ml of Sensitize (ethanol 30 ml, Sensitizer 10 ml, dH_2O 60 ml) for 10 min on a rocker. The gel was again washed in 30 % ethanol for 10 min on a rocker then in dH_2O for a

further 10 min on a rocker. The gel was incubated in Stain (Stainer 1 ml, dH_2O 99 ml) for 15 min on a rocker and then washed for less than a minute in 100 ml dH_2O. The gel was incubated in Develop (Developer 10 ml, Developer enhancer 1 drop, dH_2O 90 ml) for 4-8 min until the required staining was achieved. The developing process was stopped by adding 10 ml of Stopper directly to the previous step and incubating for 10 min on a rocker. The gel was washed in 100 ml of dH_2O for 10 min.

Chapter 3:

Testing of antibodies raised against *D. gallinae* antigen in laying hens, in an *in vitro* feeding assay

3.1 Introduction

The pragmatic approach to vaccine development using a protein antigen is to fractionate all the proteins from the pathogen of interest into subsets based on a particular property, to inject these protein fractions into the host and then to test the protective effects of the immune response generated.

This approach was used during the development of the vaccine TickGARD™ to great effect, as described in chapter 1. Cattle were immunised with crude extracts from the tick *R. microplus* that had been fractionated by differential centrifugation. This demonstrated that there was an antigen in the membrane or particle-bound fraction that generated a protective immune response in the immunised cattle, as there was a drop in tick numbers in cattle in the immunised group. The fractionation process was continued using detergents, followed by isoelectric focusing and finally gel permeation chromatography. The immune response generated by this group of proteins caused a range of effects on the ticks feeding on the immunised animals, including visible damage in an average of 96 % of the ticks in one group of cattle (Willadsen et al., 1988). Following affinity chromatography on a wheat germ lectin-Sepharose column and SDS gel electrophoresis, the glycoprotein Bm86 was then identified as the protein responsible for eliciting the protective immune response (Willadsen et al., 1989). Immunisation trials conducted with a recombinant form of the protein found it to be just as effective as the native form (Willadsen et al., 1992).

3.1.1 Immunisation study

All the fractions and recombinant proteins involved in the development of TickGARD™ were tested in large *in vivo* trials, in which immunised cattle were exposed to ticks and various aspects of the ticks were monitored, including mortality and visible damage. In this chapter, studies will be described that have been carried out with laying hens immunised with *D. gallinae* antigens. The protective effects of the antibody response generated in these studies have been measured by use of an *in vitro* feeding assay.

D. gallinae proteins in these experiments were initially fractionated based on their solubility in solutions of PBS, Tween 20, Triton X100 and urea, and in a subsequent experiment by ion exchange chromatorgraphy. This method has been used previously to fractionate *Psoroptes ovis* mites for immunisation of sheep before the animals were challenged. That study demonstrated a reduction in lesion size and mite numbers in sheep immunised with saline-soluble and Tween 20-soluble mite extracts (Smith et al., 2002). The PBS-soluble protein fraction was then sub-fractionated by ion exchange and gel filtration chromatography. The final protein fractions, when injected into sheep, reduced the numbers of *P. ovis* by more than 15-fold and lesion area by more than four-fold. However, the PBS-soluble protein fraction was subsequently shown to contain many proteins (Smith & Pettit, 2004), making protective antigen identification difficult.

In the present study, immunisations of hens with *D. gallinae* extracts were carried out at the Scottish Agricultural College (SAC) Auchincruive, and Moredun Research Institute. The purpose of these studies was to generate antibodies to be tested in the *in vitro* feeding assay to assess any increase in mite mortality caused by the immune response to one or more of the protein fractions.

3.1.2 *In vitro* feeding assay

In vitro feeding assays have been used in the study of many haematophagous parasites with a view to vaccine development, for example in hard ticks (Krober & Guerin, 2007). The *D. gallinae in vitro* feeding assay was modified from a feeding assay developed in a collaboration between Moredun Research Institute and SAC Auchincruive (McDevitt et al., 2006). The first *D. gallinae* feeding assay *in vitro* was described in 1971 (Kirkwood, 1971). During the assay development, mites were fed blood from a variety of birds such as quail and chickens, and mammals such as calves and rabbits, and the highest percentage of mites fed on quail blood. *D. gallinae* would not feed through mammalian skin or synthetic membranes and so skins from two week old quail were used as a feeding membrane (Kirkwood, 1971).

However, work more recently carried out in Moredun Research Institute (Wright et al., unpublished data), The Netherlands (Monique Mul, personal communication) and Newcastle (Harrington et al., 2009b) has suggested that synthetic membranes could be used as a feeding membrane. Previous work on feeding assays suggested that the mite feeding rates were highest through bird skins, so the skin from one day old chicks was used as the feeding membrane (Kirkwood, 1971; McDevitt et al., 2006). The assay has been used to propagate populations of *D. gallinae in vitro* (Bruneau et al., 2001) and to test antibodies raised against possible vaccine candidates (Bartley et al., 2009; Wright et al., 2009).

The assay has enabled the preliminary testing of antibodies raised against *D. gallinae* proteins to identify potential vaccine candidates, before embarking on the more technically demanding and expensive process of *in vivo* challenge studies, described in chapter 6. While the latter will be required to validate any potential vaccine, the current *in vitro* studies will allow the rapid selection of effective vaccine candidates, and enable studies on their mode(s) of action.

The assay has also been used to test the protection given by antibodies raised against two specific recombinant proteins from *D. gallinae*, tropomyosin and paramyosin, described in chapter 5.

3.1.3 Aims of chapter

The purpose of the work described in this chapter was to test the potential of native mite proteins to elicit a protective antibody response by testing the protective nature of the antibodies raised against those antigens using an *in vitro* feeding assay.

3.2 Methods

3.2.1 *D. gallinae* used in the experiments

Mixed stage and gender *D. gallinae* were collected from a commercial egg production unit and stored in a 75 cm^2 culture flask (Corning), with a vented cap. These mites were conditioned before use in the *in vitro* feeding assay by initial incubation at room temperature (~ 21 +/- 2°C) for 7 days, followed by storage at 4 +/- 1°C for 21 days (McDevitt et al., 2006).

For antigen preparation: Within 4 hr of collection, mites were taken from the culture flask, snap frozen in liquid nitrogen and stored at -80°C. These mites were at different life stages, and the majority (> 60 %) of the mites were engorged. Engorged mites were used as they would have up regulated proteins for digestion, moulting and reproduction that may be potential vaccine candidates.

3.2.2 Fractionation of *D. gallinae* proteins

Frozen mites (1 g) were homogenised and extracted sequentially with: 1) phosphate buffered saline (PBS-soluble proteins), 2) PBS containing 0.1 % v/v Tween 20 (membrane-associated proteins), 3) PBS containing 2 % v/v Triton X100 (integral membrane proteins) and 4) 10 M urea containing 2 M dithiothreitol ([DTT], insoluble proteins). Frozen mites were added to 10 ml of chilled PBS and homogenised using a mechanical homogeniser (Ultra-Turrax T25, IKA-Labortechnik) for 30 sec, on the highest power setting, rested on ice for 1 min then homogenised for a further 30 sec. The homogenised mites were then centrifuged at 25,000 x *g* for 20 min at 4°C. The supernatant (PBS extract) was stored at -80°C. The pellet was re-suspended in chilled PBS containing 0.1 % v/v Tween 20 and transferred to a Dounce homogeniser, homogenised then centrifuged as previously described. This process was repeated using a further aliquot of PBS containing 0.1 % v/v Tween 20. The supernatant was stored at -80°C. The pellet was resuspended in PBS containing 2 % v/v Triton X100 before centrifugation at 100,000 x *g* for 45 min

at 4°C. The supernatant was stored at -80°C. The pellet was re-suspended in 10 M urea and 2 M DTT and incubated at 37°C overnight. The tubes were then centrifuged at 100,000 x *g* for 20 min at room temperature (RT). A PAGE gel was prepared using an aliquot of each protein fraction. The supernatant was then stored at -80°C.

3.2.3 Immunisation of hens with the four mite protein fractions

Forty Lohmann Brown hens were obtained at 16 weeks of age and housed in a single room in ten floor pens in groups of four, under standard environmental and feeding conditions for four weeks prior to the study commencing. The hens began laying eggs at 19-20 weeks of age. At 20 weeks of age, the hens were re-divided into 10 groups of four on the basis of body weight (ensuring that the mean weights for each group were within a 100 g tolerance) and placed in floor pens under standard environmental and feeding conditions. Two pens were randomly assigned to each treatment. The hens from each treatment group were immunised with one of the four mite extracts or an adjuvant control. Each dose comprised 75 µg of mite protein at 1 µg/µl (concentration was determined using the Pierce BCA protein assay for the PBS-soluble protein, membrane-associated protein and integral membrane protein fractions as described in 2.2.1 and the 2-D Quant Kit (Amersham Biosciences) was used to determine the concentration of the urea protein fraction), 200 µg of QuilA adjuvant (1 µl), and PBS in a final volume of 300 µl. Control immunisations consisted of 200 µg of QuilA adjuvant (1 µl) in PBS (299 µl). The injections were administered into alternate breast muscles of the hen one week after they had been assigned to groups. Following the primary injection, two booster injections were administered at two week intervals.

Eggs were collected on week six (two weeks after the final boost) and the IgY antibody was extracted from the yolk as described in 2.2.4, to be used in Western blots as described in 3.2.6, and tested in the *in vitro* feeding assays as described in 3.2.7.

The experiment was carried out in accordance with the Animals (Scientific Procedures) Act 1986. The experiment was ratified by the ethics committee at the Scottish Agricultural College in Auchincruive.

3.2.4 Fractionation of PBS-soluble *D. gallinae* proteins using a Mono-Q anion exchange column

The PBS-soluble proteins were further fractionated into three fractions based on anion affinity to a Mono-Q anion exchange column HR 5/5 (GE Healthcare Amersham). *D. gallinae* were homogenised in PBS using the first part of the technique described in 3.2.2. The PBS-soluble proteins were stored at -80°C until required. The protein to be fractionated was filtered through a 0.4 µm syringe filter and then diluted 1:4 with Mono-Q buffer A as described in 2.1.6 to reduce the salt content to 30 mM. Table 3.1 details the HPLC conditions in the fractionation process.

PAGE gels were prepared as described in 2.2.8 using an aliquot of each of the protein fractions. The PAGE gels were developed using SilverQuest (Invitrogen), as described in 2.2.21. A Western blot as described in 2.2.5 was prepared using an aliquot from each protein fraction, and was probed with the antibodies raised against the PBS-soluble proteins generated as described in 3.2.3.

The immunisation doses were prepared by pooling fractions A6 to A9 for group 1, fractions A10 to B10 for group 2, and fractions B9 to B4 for group 3. The proteins were concentrated, using 10 kDa cut-off centrifugation filters (Amicon Ultra-15, Millipore), and the concentration was measured using the BCA assay (Pierce), as described in 2.2.1. These fractions were stored at -80°C in 1.5 ml tubes. Groups 1 and 2 had 75 µg of protein and group 3 had approximately 10 µg of protein per vaccination, due to low concentration of protein in the protein fractions collected from the Mono-Q column. All groups, including control, had a base of PBS and had 200 µg of QuilA adjuvant.

Table 3.1. HPLC parameters used to fractionate the PBS-soluble proteins.

Variable	Value
Column	Mono-Q HR 5/5
Flow rate	1 ml/min
Column pressure limit	3 MPa
Wavelength	280 nm
Equilibration volume	5 column volumes (cv)
Flow through tube type	18 mm
Flow through fraction size	5 ml
Empty loop volume	4 ml
Start fractionation at	0 % Buffer B (As described in 2.1.6)
End fractionation at	100 % Buffer B
Elute fraction size	1 ml
Length of gradient	20 ml
Gradient delay	2 ml
Wash with	5 cv
Re-equilibrate with	5 cv

3.2.5 Immunisation of hens with three PBS-soluble *D. gallinae* protein fractions

Twelve ISA brown chickens (20 weeks old) were obtained and were randomly allocated into groups of three hens housed in floor pens under standard environmental and feeding conditions at Moredun Research Institute. The hens were injected with the three protein fractions, prepared as described in 3.2.4, on week zero and boosted on weeks two and four. The injections were administered into alternate breast muscles of the hens. The eggs from the hens were collected six weeks after primary injection, and the IgY antibodies were extracted using the method described in 2.2.4 for use in Western blots and tested in the *in vitro* feeding assay.

The experiment was carried out in accordance with the Animals (Scientific Procedures) Act 1986. The experiment was ratified by the ethics committee at Moredun Research Institute.

3.2.6 Western Blots: Primary and secondary antibodies

Western blots were prepared using the method described in 2.2.5. The primary antibodies used in the Western blots were extracted from eggs collected six weeks after initial injection and extracted as described in 2.2.4. The IgY extracted was diluted 1:200 with Wash Buffer as described in 2.1.6. The secondary antibody was rabbit anti-chicken IgY horse radish peroxidase conjugate (Sigma), diluted 1:30,000 in Wash Buffer before use.

3.2.7 *In vitro* feeding assay and related statistics

An *in vitro* feeding assay experiment was conducted to determine the best storage conditions for chick skins to be used in the assays. The skins tested were harvested from 1 day old chicks and were stored at -20°C for two weeks, preserved in a) 100 % ethanol or b) methanol then stored at 4°C for two weeks or c) harvested on the day of the assay. The *in vitro* feeding assay was set up detailed in 2.2.6. All the mites in each chamber were scored as either alive or dead and fed or not fed. Three *in vitro* feeding assay experiments were carried out as described in 2.2.6.

The first assay tested antibodies raised against all four whole mite protein fractions and the control group as described in 3.2.3, at a dilution of one part antibody to 20 parts heparinised blood. Differences in mite mortality over the whole experiment between the four mite protein fractions and the control were statistically analysed using a proportional hazards model (Collet 1994), adjusting for the inclusion of data from the three experiments. Differences in the mortality data for the first two days of the three experiments between the four mite protein fraction treatment groups and the control group were analysed using a general linear mixed model with binomial error

and logit link function, fitting treatment group as a fixed effect and experiment as a random effect.

The second experiment used the antibodies raised against PBS-soluble and the control group where the dilution of antibody fed to the mites was changed from 1:20 to 1:1 (antibody:blood). The statistical test used for analysis of the *in vitro* feeding assay using a higher concentration of antibody was a simple logistic regression model with logit link function.

The third experiment used the antibodies raised against the three sub-fractions of the PBS-soluble protein fraction, as described in 3.2.5. The antibody preparation was diluted 1:1 with heparinised blood. The statistical test used to analyse this data was a simple logistic regression model with logit link function, which had been fitted using three replicates per group.

Statistical analyses were carried out using Genstat (Genstat, 10th edition, Lawes Agricultural Trust, VSN International Ltd, Oxford, UK).

3.3 Results

3.3.1 Initial immunisation using four protein fractions from *D. gallinae*

3.3.1.1 Initial fractionation and immunisation with four protein fractions

Figure 3.1 shows the protein profile of the sequential extraction of four protein fractions from *D. gallinae*, yielding a number of proteins with a broad range of molecular masses.

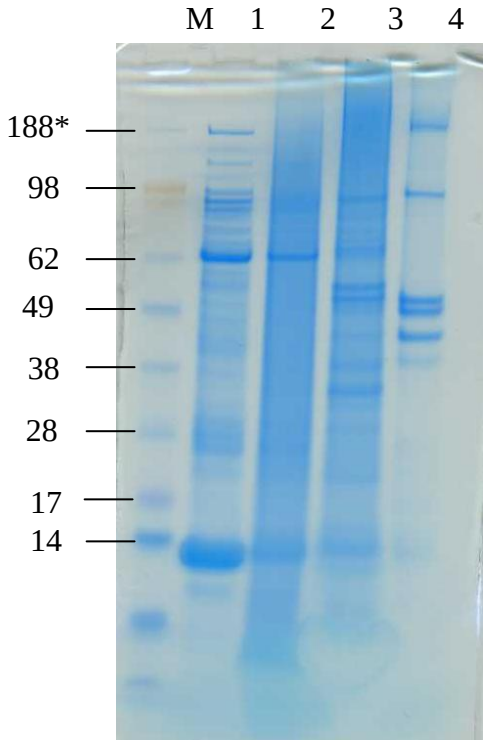


Figure 3.1. A PAGE gel showing the protein profiles of the four protein fractions to be used in the initial immunisation study. Lane M: SeeBlue plus 2 protein markers (Invitrogen). Lane 1: PBS-soluble proteins. Lane 2: Membrane-associated proteins. Lane 3: Integral membrane proteins. Lane 4: Insoluble proteins. The gel was stained with SimplyBlue (Invitrogen) stain. *Molecular mass (kDa)

3.3.1.2 Initial immunisation seroconversion

Figure 3.2 is a scan of a Western blot prepared with the four *D. gallinae* protein fractions and probed with the antibody, extracted from egg yolk, raised against each fraction, and the control antibodies as described in 3.2.3. As can be seen, antibodies were raised against proteins with a broad range of molecular masses (lanes 1-4). A small number of proteins were detected in the control blot (lanes 5-6), two of which were probably subunits of IgY (heavy chain ~ 65 kDa, light chain ~ 21 kDa), since the mites used in the protein extraction had recently fed and would likely have IgY in their gut.

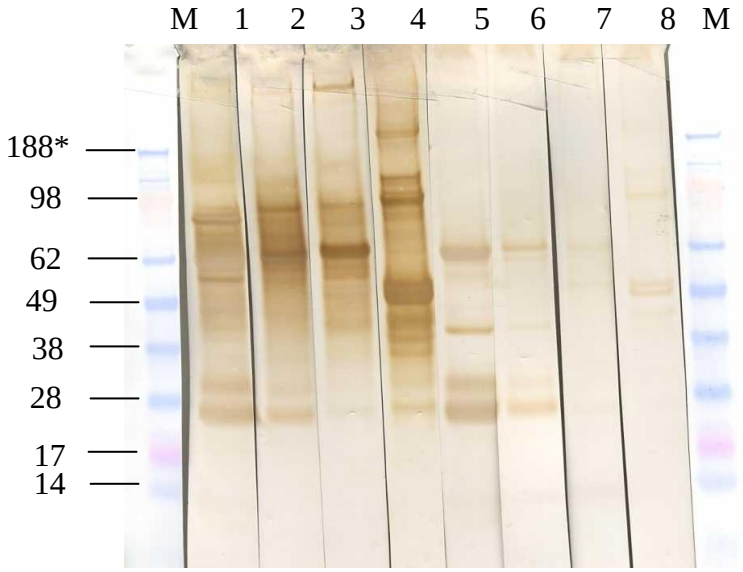


Figure 3.2. Western blots of mite proteins probed with antibodies raised against the four *D. gallinae* protein fractions. Lane M: SeeBlue plus 2 protein markers (Invitrogen) Lane 1: PBS-soluble proteins, probed with antibodies raised against PBS-soluble proteins. Lane 2: Membrane-associated proteins, probed with antibodies raised against membrane-associated proteins. Lane 3: Integral membrane proteins, probed with antibodies raised against integral membrane proteins. Lane 4: Urea-soluble proteins, probed with antibodies raised against urea-soluble proteins. Lane 5: PBS-soluble proteins, probed with antibodies raised in the control group (adjuvant only). Lane 6: Membrane-associated proteins, probed with antibodies raised in the control group. Lane 7: Integral membrane proteins, probed with antibodies raised in the control group. Lane 8: Urea-soluble proteins, probed with antibodies raised in the control group. *Molecular mass (kDa).

3.3.2 Second immunisation with three fractions of the PBS-soluble proteins

3.3.2.1 PBS-soluble protein fractions

The graph in figure 3.3 shows the ultraviolet absorption profile (at 280 nm) against volume of the protein fractions eluted from the Mono-Q column. Fraction A1 contains the unbound proteins, i.e. those of neutral charge under anion conditions, or proteins that did not bind due to a lack of free ions. Fractions A2 to B4 contained proteins that dissociated from the column at increasing NaCl concentrations. As can be seen, there was a large peak in absorbance in fractions A8 to A10.

Figure 3.4 is a scan of a PAGE gel developed with silver stain, as described in 3.2.5, that shows the protein profiles after fractionation of the PBS-soluble proteins using anion exchange.

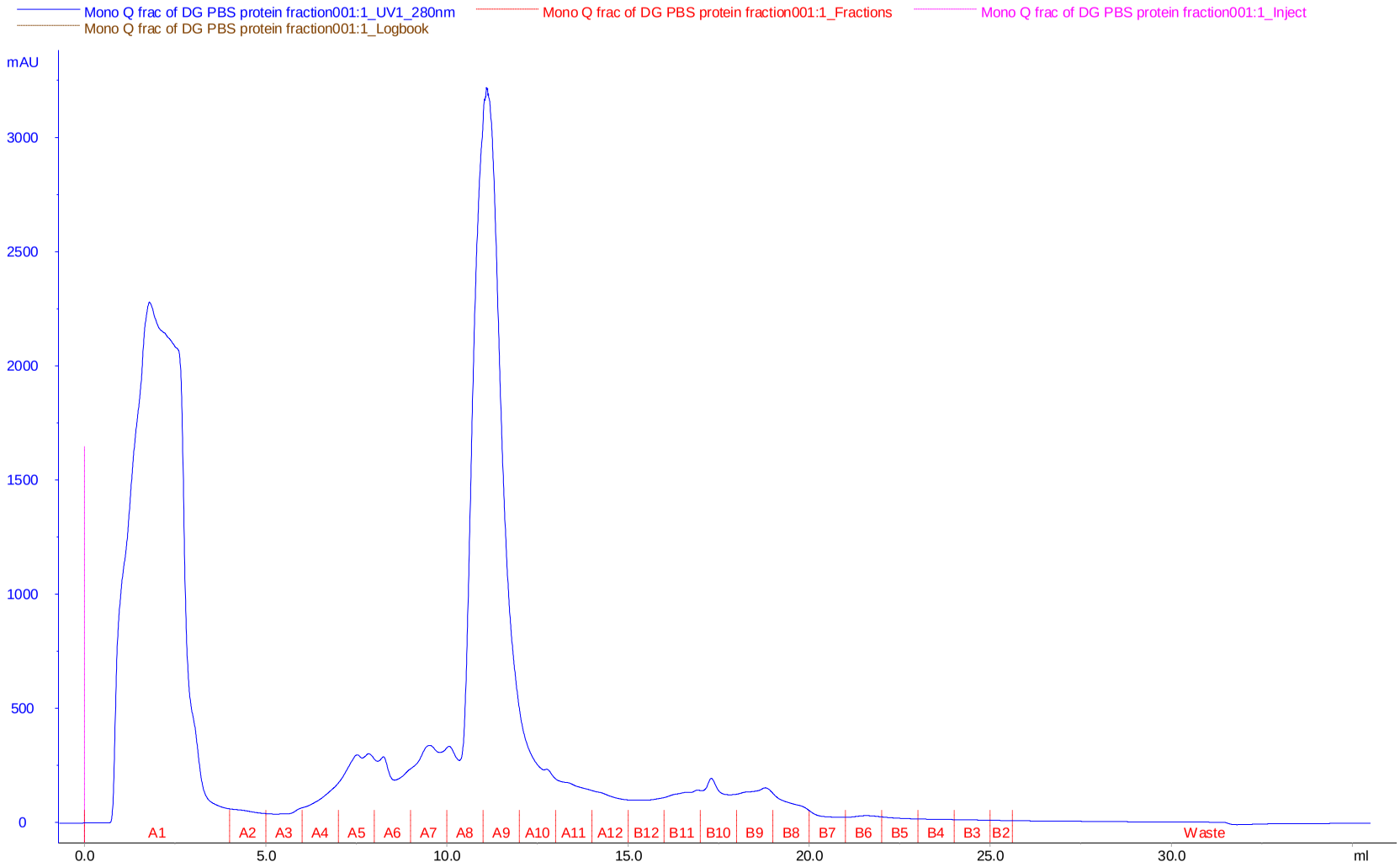


Figure 3.3. A graph of the UV absorbance as proteins disassociate from the Mono-Q column with increasing NaCl concentration. The salt concentration increases from 0 M to 1 M . The salt gradient increase from 0 to 100 % from fractions A2 to B4 respectively .

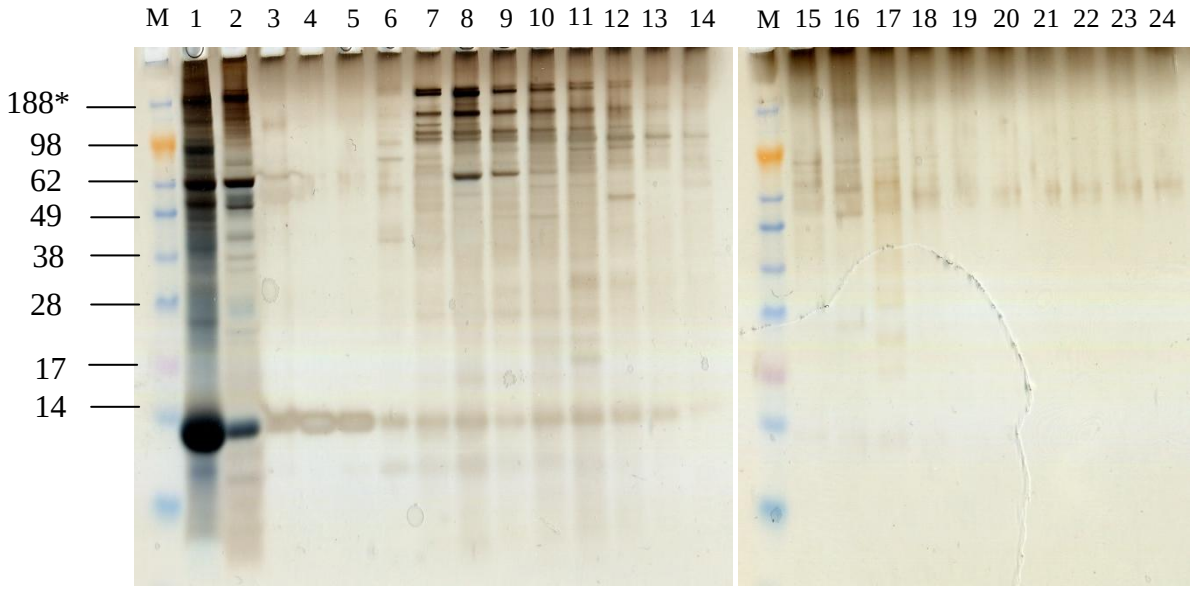


Figure 3.4. A polyacrylamide gel showing the protein profiles of the sub fractionated PBS-soluble proteins that were injected into hens. Lane M: SeeBlue plus 2 protein markers (Invitrogen). Lane 1: PBS protein fraction before Mono-Q column fractionation. Lane 2: Unbound fraction A1. Lane 3: Fraction A2. Lane 4: Fraction A3. Lane 5: Fraction A4. Lane 6: Fraction A5. Lane 7: Fraction A6. Lane 8: Fraction A7. Lane 9: Fraction A8. Lane 1: Fraction A9. Lane 11: Fraction A10. Lane 12: Fraction A11. Lane 13: Fraction A12. Lane 14: Fraction B12. Lane 15: Fraction B11. Lane 16: Fraction B10. Lane 17: Fraction B9. Lane 18: Fraction B8. Lane 19: Fraction B7. Lane 20: Fraction B6. Lane 21: Fraction B5. Lane 22: Fraction B4. Lane 23: Fraction B3. Lane 24: Fraction B2. *Molecular mass (kDa).

Figure 3.5 is a scan of the Western blots prepared using the 23 protein fractions from the Mono-Q anion exchange column and probed with the antibodies generated against the unfractionated PBS-soluble protein fraction from the initial immunisation. The blot was developed using ECL and shows immunoreactive bands at a broad range of molecular masses from 188 kDa to ~28 kDa from fraction A5 to fraction B4.

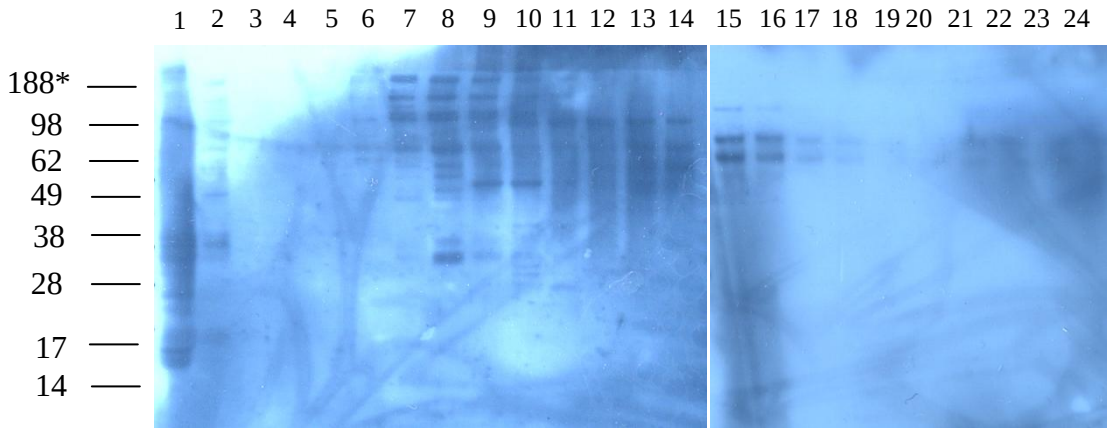


Figure 3.5. A Western blot probing all 23 fractions eluted with antibodies raised against the PBS-soluble protein in the initial immunisation. Lane 1: protein applied to column. Lane 2: Fraction A1, unbound to column. Lane 3: Fraction A2. Lane 4: Fraction A3. Lane 5: Fraction A4. Lane 6: Fraction A5. Lane 7: Fraction A6. Lane 8: Fraction A7. Lane 9: Fraction A8. Lane 1: Fraction A9. Lane 11: Fraction A10. Lane 12: Fraction A11. Lane 13: Fraction A12. Lane 14: Fraction B12. Lane 15: Fraction B11. Lane 16: Fraction B10. Lane 17: Fraction B9. Lane 18: Fraction B8. Lane 19: Fraction B7. Lane 20: Fraction B6. Lane 21: Fraction B5. Lane 22: Fraction B4. Lane 23: Fraction B3. Lane 24: Fraction B2. *Molecular mass (kDa).

3.3.2.2 Immunisation with sub-fraction of PBS-soluble proteins

Hens had been immunised with four protein fractions based on anion exchange. The fractions pooled were: group 1 fractions A6 to A9, group 2 fractions A10 to B10 and group 3 fractions B9 to B4. Figure 3.6 is a scan of a Western blot prepared with PBS-soluble proteins and probed with the antibodies raised against the fractionated PBS-soluble protein fraction. The antibodies generated against the three pooled PBS-soluble protein fractions, groups 1, 2 and 3 as described above, were used to probe the PBS-soluble protein fraction and then compared to a Western blot that probed the PBS-soluble protein fraction with antibodies generated in the experiment described in 3.2.3.

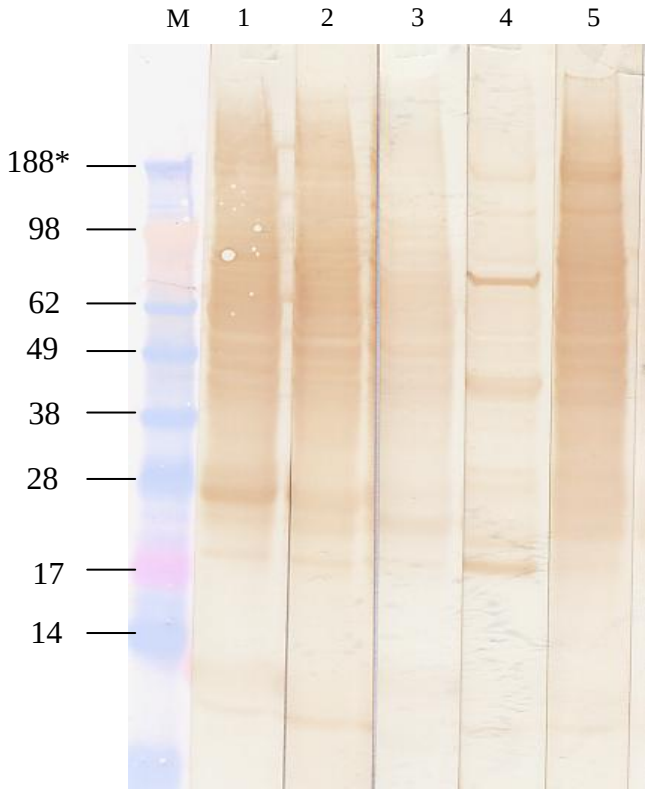


Figure 3.6. A scan of a Western blot prepared with the PBS-soluble protein fraction (prepared as described in 3.2.2) and probed with the antibodies raised against the three fractionated PBS-soluble protein fractions. Lane M: SeeBlue plus 2 protein markers (Invitrogen). Lane 1: PBS-soluble proteins probed with antibodies generated against protein from group 1. Lane 2: PBS-soluble proteins probed with antibodies generated against protein from group 2. Lane 3: PBS-soluble proteins probed with antibodies generated against protein from group 3. Lane 4: PBS-soluble proteins probed with antibodies generated against protein from the control group. Lane 5: PBS-soluble proteins probed with antibodies raised against the PBS-soluble protein fraction. *Molecular mass (kDa).

3.3.3 *In vitro* feeding assay

3.3.3.1 Preliminary testing

This experiment was performed to test a variety of membranes through which the mites could feed during the *in vitro* feeding assays. The mites that fed through ethanol preserved skins had the highest percentage feeding and the second lowest percentage mortality as seen in table 3.2, which shows the percentages of mites that had or had not fed and were alive or dead. This suggested that the chick skins could be preserved in ethanol for use in the *in vitro* feeding assays.

Table 3.2. Percentages of mites that had or had not fed and that were alive or dead.

Treatment	Dead/Fed (%)	Dead/Not Fed (%)	Live/Fed (%)	Live/Not Fed (%)
Fresh	1.65	8.24	28.57	61.54
Frozen	2.2	2.2	27.47	68.13
Ethanol	1.67	4.34	31.78	62.21
Methanol	6.73	5.29	14.42	73.56

3.3.3.2 Testing antibodies raised against the four mite protein fractions

There was an uncontrollable variability in the numbers of mites feeding in each chamber, which meant that various numbers of mites progressed into the mortality monitoring phase of the experiments. Table 3.3 shows the numbers of mites that fed in each group. Because the number of mites feeding was variable for different groups and experiments, mortality was expressed as a cumulative percentage to allow unbiased summary comparison between treatment groups and each experiment. However, while estimates will be unbiased, the power to test the effect of the four mite protein fractions will vary marginally with the total number of feeding mites per fraction over the three experiments. Figure 3.7 is a graph of the cumulative percentage mite mortality against time, which differed between the four groups injected with mite protein over the course of the experiment. The percentage mortality was highest when the mites were fed antibodies raised against the PBS-soluble protein fraction, with cumulative percentage mortality (across three experimental repeats) of 16.4 % after 24 hr, compared to 10.9 % for the controls. The cumulative percentage mortality in the PBS-soluble treatment rose from 16.4 % to 34.8 % after 14 days, whereas the cumulative percentage mortality in the control mites rose from 10.9 % to 27.3 % after 14 days. The proportional hazards model showed that there was a statistically significant difference in mortality rates between the five treatments ($p = 0.043$). Parameter estimates showed that the anti-PBS protein fraction antibodies caused the highest mean mortality compared to the control group, but that this difference was not statistically significant when taken over the course of the experiment.

Table 3.3. The number of feeding mites in each group for the three experiments.

Group	Experiment 1	Experiment 2	Experiment 3
Control	98	24	43
PBS-soluble	58	100	92
Membrane-associated	57	72	61
Integral membrane	104	24	28
Insoluble	78	189	51

Focusing on the data from the first two days of the experiment (figure 3.7), a difference was observed between the gradient, or mortality rates, of the PBS-soluble protein group and control group, with the rate for the PBS-soluble protein group being consistently greater than that for the control group. This, however, was not the case from day three onwards. The generalised linear mixed model showed that there was a significant difference in the mean mortality in the first two days between the five groups (p = 0.016). Parameter estimates showed that the anti-PBS-soluble protein fraction antibodies caused the highest mean mortality compared to the control, and that this effect was statistically significant (p = 0.013).

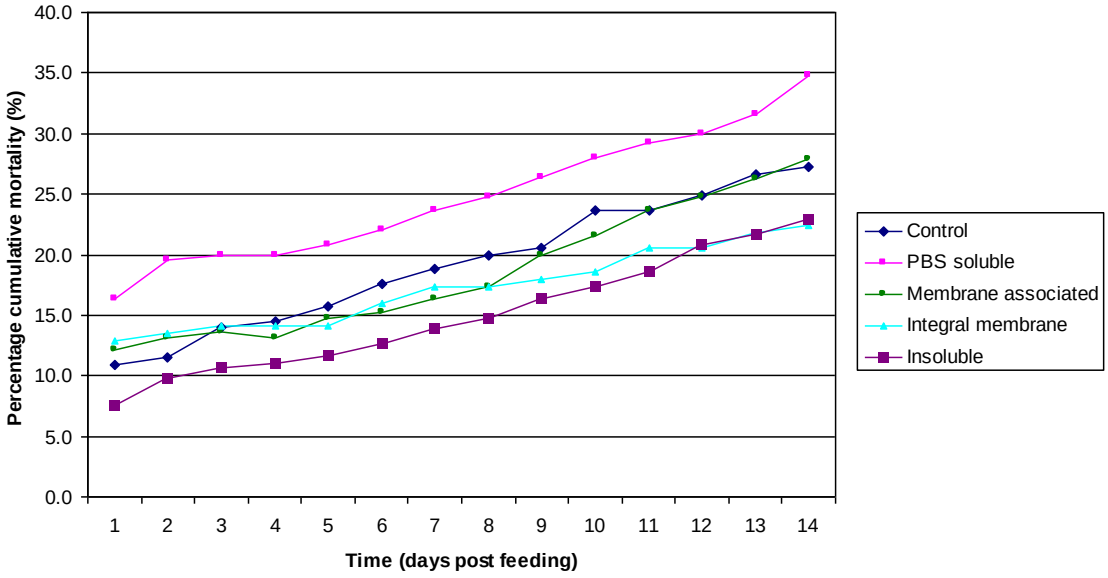


Figure 3.7. Cumulative percentage mortality of *D. gallinae* fed heparinised chicken blood enriched with antibodies raised against four mite protein fractions and adjuvant-only (control). Mortality was monitored daily over a 14 day period following feeding. Each point represents cumulative mean percentage mortality and is derived from data recorded in three independent experiments. The range of the Standard Error of the Mean over the 14 days were Control 0.55-6.18; PBS-soluble group 3.36-8.04; Membrane-associated group 1.80-6.22; Integral membrane group 0.93-5.00; Insoluble group 2.54-3.96.

3.3.3.3 Testing concentrated antibodies raised against PBS-soluble proteins

The results described in this section were derived from an *in vitro* feeding assay. A higher concentration of the antibodies generated against the PBS-soluble protein fraction, as described in 3.2.3, was mixed with fresh hen blood and fed to the mites. Table 3.4 shows the number of mites that fed in each experiment, as can be seen, there was not much variation in the numbers in the test group. The number of mites that fed in the control group varied to a higher degree. Figure 3.8 is a graph of the cumulative percentage mortality of the mites in the assay against time.

Table 3.4. The number of feeding mites in each group for the three experiments.

Group	Experiment 1	Experiment 2	Experiment 3
Control	68	53	43
PBS-soluble	65	66	52

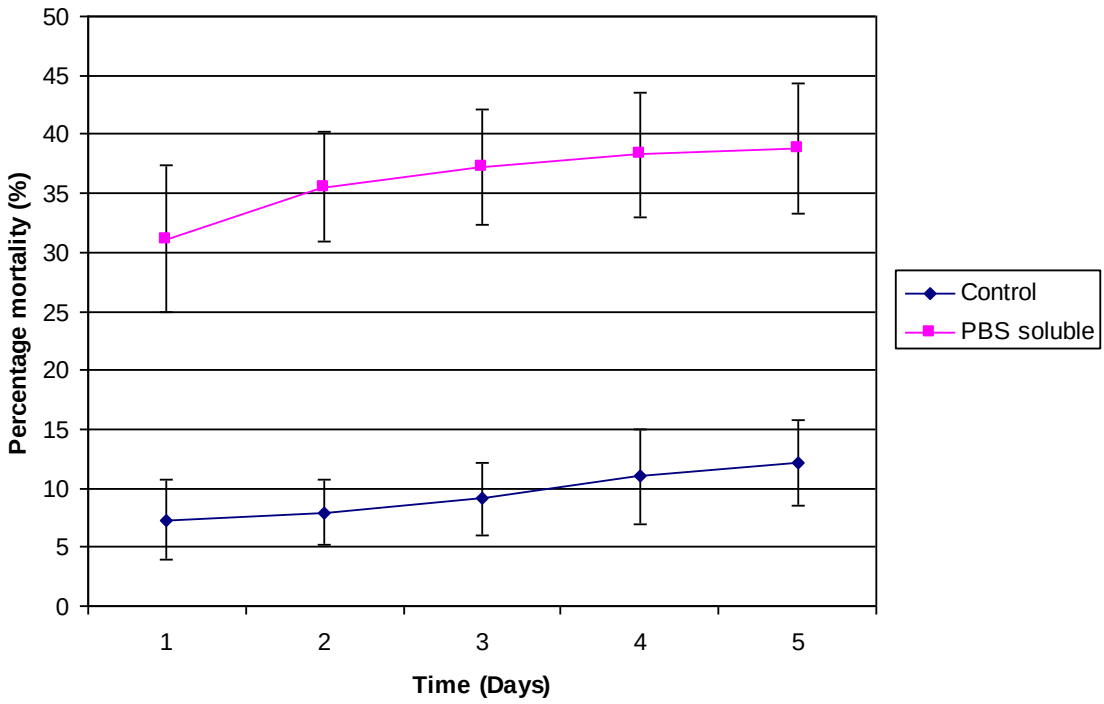


Figure 3.8. A graph of the cumulative percentage mortality of *D. gallinae* fed antibodies raised against PBS-soluble protein fraction and antibodies raised against adjuvant only (control). Mortality was monitored daily over a five day period following feeding. Each point represents cumulative mean percentage mortality and is derived from data recorded in three independent experiments. Error bars show the standard error of the mean.

The mortality of the mites fed antibodies from the PBS-soluble protein fraction group was higher than that of the controls. The titre of the antibody was increased in this experiment, compared to the initial experiment with all five groups. As can be seen, the mortality of the mites increased when the concentration of antibody increased in the test group but not the control group. From Figure 3.7, the mortality of the mites fed antibodies from the PBS-soluble group was 16.4 % after one day and increased to 20.8 % after five days, compared to 31 % to 39 % from day one to five respectively during the second experiment, with a higher antibody concentration. The

mortality of the control group in each experiment was roughly the same from 7.5 % after one day to 1.6 % after five days for the first experiment with a lower concentration of antibody and 7 % to 12 % for the second experiment with a higher concentration of antibody. The cumulative percentage mortality of the mites fed antibodies from the PBS-soluble fraction more than doubled in this experiment compared to those fed the same antibodies at a lower concentration in the previous experiment, as described in 3.3.3.2. A simple logistic regression model showed that there was a significant difference between the two groups after five days, with a *p* value of 0.017.

3.3.3.4 Testing antibodies raised against three PBS-soluble protein fractions separated by ion exchange chromatography

Figure 3.9 is a graph showing the percentage mortality of *D. gallinae* fed antibodies raised against the three pools of the PBS-soluble protein fractions after fractionation by Mono-Q column. As can be seen, the pooled protein fraction 1 caused the highest mortality in the mites. All the protein fractions caused a higher mortality in the mites than the controls. This data was collected from a single assay, as replication was restricted by the availability of suitably conditioned mites.

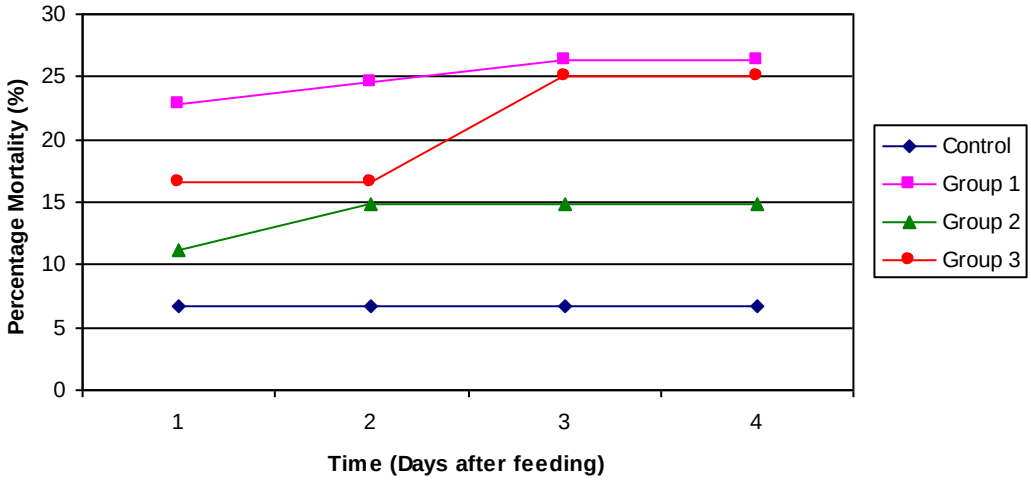


Figure 3.9. A graph of the percentage mortality of *D. gallinae* fed antibodies raised against the fractionated PBS-soluble protein fraction and antibodies raised against adjuvant only (control). Mortality was monitored daily over a four day period following feeding. The data presented in this graph was collected from a single assay where each data point represented the percentage mortality of the fed mites for each group on each day. The number of mites fed in each group was variable: Control group had 12 fed mites, Group 1 had 57 fed mites, Group 2 had 27 fed mites and Group 3 had 12 fed mites. The hens in Group 1 were injected with protein from fractions A6 to A9, Group 2 were injected with protein from fractions A10 to B10, Group 3 were injected with protein from fraction B9 to B4 and Control group were injected with the QuilA adjuvant only.

3.4 Discussion

The experiments described in this chapter demonstrate that when antibodies were raised against *D. gallinae* protein extracts and tested in an *in vitro* feeding assay, the antibodies raised against some of the mite proteins significantly increased the mite mortality in the test group when compared to the control group. These protective, anti-mite, antibodies were raised against proteins from the PBS-soluble protein fraction that have not yet been identified. During the first two days after feeding, the mean mortality of the mites from the PBS group was significantly higher ($p = 0.013$) than that of the control group (figure 3.7). This mortality may be attributable to antibodies directed towards a single antigen, or to multiple antigens, important to the normal functioning and viability of the mite. These antigens appear to be present predominantly in the PBS-soluble protein fraction, and these PBS-soluble proteins therefore represent a likely source of vaccine candidate(s) which require identification and characterisation.

The increased mortality of fed mites was modest but it should be noted that during the development of TickGARD™, which was previously an effective commercial vaccine against ticks, the initial effects of vaccination with crude tick extracts were also comparatively modest. As the protein component of the vaccine was reduced in complexity, the efficacy increased up to 40 % (Johnston et al., 1986), and to 90 % by use of either pure native or recombinant Bm86 (Willadsen et al., 1995). It is also notable that the increased mortality seen in this experiment resulted after a single bloodmeal, whereas in nature the mites would feed repeatedly, potentially generating an enhanced cumulative effect of the antibodies generated against the PBS-soluble protein fraction on the mortality of the mites.

Numerous proteins with a broad range of molecular masses were recognised by IgY from vaccinated hens, and the profiles of proteins recognised differed between the mite protein extracts used for immunisation (figure 3.2). When comparing the reactivity of the vaccinated groups to the reactivity of the adjuvant-only groups, there was a marked difference in the number and intensity of the bands. Some of the bands

detected in both the vaccinated and control groups represented the two IgY subunits. Bands of approximately 62 kDa and 25 kDa in lanes 1 and 6 and, to a lesser extent, 2 and 7, most likely represent the IgY subunits present in the mites' bloodmeal that were detected with the anti-IgY secondary antibody.

The differences between the profile of the PAGE gel (figure 3.1) and the banding patterns of the Western blot (figure 3.2) could be explained by the co-purification of chicken proteins present in the mite's gut that were injected into the chicken. Because of MHC restriction, the chicken would not mount an antibody against "self" proteins. The immunogenic proteins that create bands on the Western Blot might not be very abundant.

Chick skin was used as a feeding membrane during the development of the assay and so was used throughout the experiments (McDevitt et al., 2006). To find a suitable storage method for the chick skin, an assay was carried out using skin stored in various ways. The mites fed best through the skins that had been stored at 4°C in ethanol, which was an easy method for storage.

The feeding assays carried out using antibodies raised against all four mite protein fractions demonstrated that the antibodies raised against the PBS-soluble protein fraction did increase the mortality of the mites significantly when compared to the controls two days after feeding. The proteins present in the PBS-soluble protein fraction have not yet been identified. Immunoreactive bands have been found when antibodies against tropomyosin and paramyosin were used to probe the PBS-soluble protein fraction. The other mite protein fractions did not seem to generate antibodies that increased the mortality of the mites. These protein fractions could contain potential vaccine candidates, but due to a very low abundance of the protein in the immunisation, a low antibody response was generated.

The PBS-soluble protein fraction was further fractionated, based on the charge of the proteins, by a Mono-Q column. The antibodies generated against these proteins were compared to the antibodies generated against the whole PBS-soluble protein fraction

(figure 3.6). The banding pattern from the Western blot showed differences between the immunoreactive proteins in the three fractionated protein groups.

An *in vitro* feeding assay was performed using the antibodies generated against the three fractionated PBS-soluble protein fractions. The mortality of all the test groups was higher than that of the control group (figure 3.9). This assay was not repeated, so no statistical analysis could be carried out. The mortality of the control groups was lower in the fractionated PBS-soluble protein assay at 6.7 % for every time point (figure 3.9) than in the PBS-soluble protein fraction assay, where the mortality in the control group went from 7.3 % to 10.9 % from day one to four (figure 3.8). Group 1 had the highest mortality of the mites fed antibodies raised against the protein of the fractionated PBS-soluble proteins (figure 3.9, 26.6 % by day four). This was lower than the mortality induced by the whole PBS-soluble protein fraction (figure 3.8, 38 % by day four). When compared to the mortality in the control group, the mortality of all the groups in the fractionated PBS-soluble protein assay was lower than the mortality in the test group fed antibodies against the complete PBS-soluble protein fraction. However, there was only data from a single assay for the fractionated PBS-soluble protein fractions, where the number of mites feeding in the assay was low, with only 15 mites feeding in the control group and 12 in group 3.

The number of mites feeding was variable from chamber to chamber in all of the experiments. In some of the chambers, over 50 % of the mites fed, whereas in others none were observed to feed. This may have depended upon the part of the chick's body from which the skin was derived, and factors such as skin thickness or attached mesoderm may have influenced the mite's ability to penetrate and feed. The method of storing the skins may also have contributed to the low feeding rates. Ethanol was chosen as a preservative based on the results of a single assay. Assays using a similar technique stored the chick skins at -20°C to produce a high feeding rate. However, there appeared to be some inconsistencies in the reporting of the results (Arkle et al., 2009). A kairomone has been identified in hen skin that is an attractant to mites (Zeman, 1988). This kairomone may have differed in concentration on the skin used

as a feeding membrane of the different chambers, affecting the overall feeding of mites in each replicate.

Some of the mites in the assay took a bloodmeal and then died. The chicken blood present in the legs could have seeped through damaged sections of the mites' gut. Figure 3.10 is a photograph of a dead mite that had internal damage leading to chicken blood spilling into the legs of the mite, as can be seen by red spots in the mite legs.

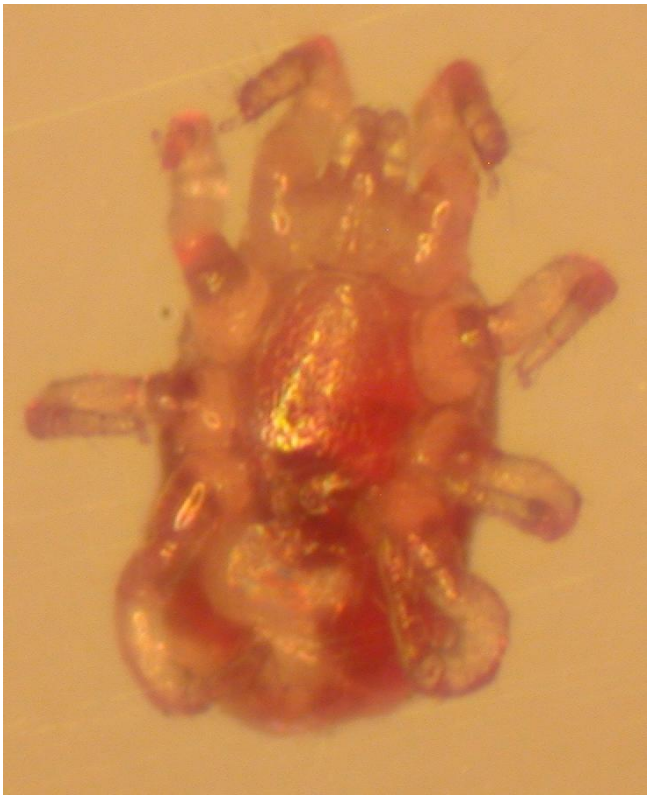


Figure 3.10. A photograph of a dead mite after taking a bloodmeal containing antibodies raised against the PBS-soluble protein fraction. This mite had suffered internal damage causing blood from the gut to seep into the mite's legs.

The efficacy of PBS-soluble proteins as vaccine candidates against mites differs somewhat from results for the tick *R. microplus* where membrane-associated proteins gave the most promising results in reducing populations as discussed above (Willadsen et al., 1995). The data presented here are, however, in agreement with work performed previously, using a similar approach to identify protective antigens

from *P. ovis*. PBS-soluble components were more protective than any other extracts, eliciting a 15-fold reduction in mite burden and four-fold reduction in scab lesion when sub-fractionated PBS-soluble protein extracts were used in *in vivo* vaccine trials (Smith et al., 2002).

In summary the experiments described in this chapter indicate that the immunised hens generate antibodies against proteins in the PBS-soluble protein fraction that have a deleterious effect on mite mortality.

Chapter 4:

Identification of vaccine candidates by the bioinformatic analysis of *Dermanyssus gallinae* transcriptome

4.1 Introduction

Studies were performed to identify native vaccine candidates, as described in chapter 3. Crude protein fractions were injected into laying hens and, by use of an *in vitro* feeding assay, it was established that the mites fed antibodies raised against the PBS-soluble protein fraction from *D. gallinae*, had a higher mortality than those fed antibodies from the control group (Wright et al., 2009). Although antibodies raised against the PBS-soluble protein fraction were clearly effective at increasing the mite mortality *in vitro*, as a practical vaccine it has disadvantages because the production of the antigen is time consuming and requires a large quantity of mites as starting material. A commercial vaccine against *D. gallinae* has to be produced using a cost-effective and rapid method. Recombinant proteins have been used to successfully immunise host animals against parasitic species; for example TickGARD™ was developed using the recombinant form of Bm86 to protect cattle from the tropical tick *Rhipicephalus microplus* (Rand et al., 1989). To obtain a single, or cocktail of, recombinant proteins, a detailed analysis of *D. gallinae* proteins is required to identify putative vaccine candidates through inferred critical function or orthology to known antigens in other species.

At the start of this project there were only five sequences for *D. gallinae* on any of the publically available online genomic or proteomic databases. Of the sequences present, tropomyosin (Nisbet et al., 2006a) was the only non-ribosomal sequence. During this project new sequencing technology, Roche 454 and Illumina Solexa, became available that allowed large numbers of sequences to be generated relatively cheaply, allowing the discovery of much larger volumes of gene sequences.

4.1.1 Expressed sequence tag (EST) database generated from fed *D. gallinae*

Expressed sequence tag (EST) databases can be quickly generated and provide portions of the sequence of genes encoding proteins that are expressed in an

organism. The proteins translated from these mRNAs may be identifiable as vaccine candidates and drug targets, as described for the parasitic filarial nematode *Onchocerca volvulus*, where EST databases were generated using larvae at those stages targeted by the host immune response to identify potential targets for vaccine development. Contiguous sequences (contigs) that were homologous to proteases such as cathepsin L, B and a metalloproteinase were thus identified in the EST databases as vaccine candidates (Lizotte-Waniewski et al., 2000).

An EST database can be specifically constructed to study the expressed proteins in a particular tissue type or life cycle stage depending on the starting material from which the complementary DNA (cDNA) is made. To build a database, Sanger sequencing single pass technology (Sanger et al., 1977) is used to sequence individual clones of a cDNA library. The individual sequences are built into larger contigs based on overlapping DNA sequence. Homologous genes and their inferred protein products are identified using a basic linear alignment search tool (BLAST) (Altschul et al., 1994) analysis with the partial sequence of the EST. This analytical technique has been used to find homologues of proteins that are potential vaccine candidates in the larval stage of *Brugia malayi* (Blaxter et al., 1996) and *Psoroptes ovis* (Kenyon et al., 2003). The analysis of EST datasets can now be carried out quickly using computer software inputting raw EST sequences and returning assembled annotated ESTs. ESTExplorer is an example of an automated platform that can accept two forms of input raw EST sequences or pre-assembled contigs. Software is used to assign annotation to the EST such as blastx and BLAST2GO, which assigns protein function based on Gene Orthology (GO), from the National Centre for Biotechnology Information (NCBI), KOBAS, which maps the contigs to biochemical pathways from the Kyoto Encyclopaedia of Genes and Genomes database (KEGG) and InterProScan (Quevillon et al., 2005), which recognises protein signatures (Ranganathan et al., 2009).

The contigs in an EST database are a representative sample of all the genes expressed. For identification of potential vaccine candidates it may be necessary to refine the dataset to identify genes involved in essential physiological processes,

such as feeding. Two techniques were used in the experiments described in this chapter to identify differentially expressed genes that could be vaccine candidates. The first used suppression subtractive hybridization (SSH) to remove all the common genes between two samples of cDNA, as described in 4.2.2. The second technique used Illumina Solexa sequencing to generate millions of sequences of approximately 25 base pairs, which were compared to a Roche 454 database generated, as described in 4.1.3. The fold change between two samples for each RNA transcript can be calculated, as described in 4.1.4. In both cases, these technologies were used to identify genes up-regulated by feeding, as these genes are likely to encode proteins critical to mite development, reproduction and survival and which are likely to have been expressed in gut tissue, which is accessible to the host immune response. As previously stated, the *R. microplus* protein Bm86 is a gut-bound glycoprotein that has previously been successfully used as the antigen in a commercial vaccine. Although it is not highly expressed, the protein can generate a protective immune response that significantly damages the tick, causing a reduction both in tick body weight and in cumulative egg weight of 31 % and 77 % respectively (Rand et al., 1989). As previously stated the vaccine causes an overall vaccine effect of 90 % when the parameters weight of engorged ticks, number of engorged ticks and weight of eggs were taken together (Willadsen et al., 1995).

4.1.2 Suppression subtractive hybridisation

To study the differences in gene expression between fed and starved mites an SSH cDNA library was constructed. The library was made by subtracting cDNA made from RNA extracted from starved mites, from cDNA made from RNA extracted from fed mites. This removed the common genes from the two libraries and left the unique genes expressed in fed mites (Diatchenko et al., 1996). These unique genes would therefore have been up-regulated and would be potential vaccine targets.

SSH has been used to target groups of genes that were up-regulated in fed *Psoroptes ovis* (McNair et al., 2010) and *Ixodes scapularis* (Xu et al., 2005). *P. ovis* total RNA was extracted and an SSH library was constructed from fed and starved populations

of mites. The genes up-regulated in the fed mites were homologous to known allergens, enzymes and secretory proteins from the salivary gland of the tick (McNair et al., 2010). An SSH library was made from cDNA libraries constructed from mRNA extracted from the salivary glands of fed and starved *I. scapularis* females. Five hundred colonies from this SSH library were sequenced, and 20 individual clones were identified. Primers specific to the 20 clones were used to amplify the genes from the two starting fed and starved cDNA libraries. Differential expression of ten genes was determined; two of which were entirely absent from the starved cDNA library. Of the 10 proteins, seven were of unknown function but had previously been described in *Anopheles gambiae*, the salivary gland of *I. scapularis* and from the mammary gland of the mouse *Mus musculus*. The three proteins of known function were thioredoxin-1, a secreted growth factor characterised in *Mesobuthus cyptius* (scorpion), a heat shock protein characterised in *I. scapularis* and a proclotting enzyme characterised in *Tachypleus tridentatus* (Japanese horseshoe crab). These proteins can be fully characterised and their successful expression could make them potential vaccine candidates (Xu et al., 2005).

4.1.3 Roche 454 sequencing of *D. gallinae* transcriptome

Roche 454 sequencing uses an emulsion-based method to isolate single DNA molecules *in vitro*, and then acquires the genetic sequence by use of pyrophosphate-based sequencing. Roche 454 sequencing technology can produce more than 25 megabases of sequence for every four hour run, with an accuracy of 99 %, whereas classical Sanger sequencing technology provides approximately 67 kilobases of sequence in a one hour run. Roche 454 technology does not require the use of bacteria to grow individual clones as it can use purified RNA as a starting material (Margulies et al., 2005).

The technology has progressed in the past five years and now Roche 454 sequencing can produce sequences with an average of length of 250 base pairs for approximately

400,000 reactions per run, giving about 100 megabases (The GenePool, Edinburgh University). These sequences are built into contigs as described for EST databases.

4.1.4 Illumina Solexa

Illumina Solexa sequencing (Bennett et al., 2005) can produce 3,000 megabases of sequence per run, which relates to 60 million individual sequences of 50 bases. Both DNA and RNA can be used as a starting material for the sequencing procedure.

Illumina Solexa sequencing was used to compare the expression levels of genes in two populations of mites, one of which had recently fed and a second that had been starved. Pre-processing of the RNA left an adapter sequence followed by 17 gene specific base pairs that can be identified by Sanger sequencing and then therefore the number of each gene present can be counted (Morrissy et al., 2009). The identity of these sequences can then be allocated by comparing them to the Roche 454 database.

4.1.4 Aims of chapter

The aim of this chapter was to identify possible vaccine candidates by inferred critical function or homology to known vaccine candidates from other species, using four sequencing techniques; two EST databases were generated from cDNA libraries, the first from total RNA extracted from *D. gallinae* and the second from a suppression subtractive hybridization library generated from fed and starved mites. A third EST database was generated by Roche 454 sequencing using total mite mRNA and Illumina Solexa sequencing was then used to compare the levels of gene expression between samples of fed and starved mites.

4.2 Methods

4.2.1 *D. gallinae* used for RNA extraction

The *D. gallinae* collected from a commercial egg production unit were of mixed life stages and gender. The majority (> 60 %) of the mites were recently engorged. For RNA preparation, the mites were snap frozen in liquid nitrogen within 4 hr of collection and stored at -80°C until required.

Starved *D. gallinae* used for SSH and Illumina Solexa sequencing were conditioned by initial incubation at room temperature (RT) (~ 21°C +/- 2°C) for 7 days, followed by storage at 4°C +/- 1°C for 21 days (McDevitt et al., 2006). At the end of the conditioning period the mites were snap frozen in liquid nitrogen and stored at -80°C until required.

4.2.2 RNA extraction

A mortar and pestle were cooled to -80°C. The mortar was filled with liquid nitrogen and a known weight of *D. gallinae*, prepared as described in 4.2.1, was added. The pestle was used to grind the mites until the liquid nitrogen had evaporated. TRIzol (Invitrogen) was added at 2 ml per 100 mg of mites. The TRIzol reagent was mixed with the powdered mites and frozen. The pestle was used to break apart the frozen TRIzol. The TRIzol/*D. gallinae* sample was ground and then mixed until it had thawed. Aliquots (1 ml) of the sample were put into RNase/DNase free 1.5 ml tubes (Axygen). They were incubated for 5 min at RT. Chloroform (Sigma)(200 µl) was added to each aliquot then inverted to mix, followed by 3 min incubation at RT. The samples were then centrifuged at 12,000 x *g* for 15 min at 4°C. A pipette was used to carefully remove the supernatant, the aqueous fraction, into a new 1.5 ml tube. Isopropanol (Fisher)(0.5 ml) was added to the 1.5 ml tube to precipitate the RNA, which was then inverted to mix and incubated at RT for 10 min. The 1.5 ml tube was centrifuged for 10 min at 12,000 x *g* at 4°C. A needle and syringe were used to remove and discard the supernatant to decrease the chance of disturbing the pellet of

RNA. Then 1 ml of 75 % ethanol was added and incubated for 5 min at RT. The 1.5 ml tube was centrifuged for 5 min at 7,500 x *g* at 4°C. All the supernatant was removed and the pellet was re-suspended in 50 µl of RNase free water (Promega).

An agarose gel was prepared, as described in 2.2.9, on which 2.5 µl of the RNA was examined for integrity by electrophoresis. RNasin (Promega) [2.5 µl (100 units)], an RNase inhibitor, was added to the RNA. The concentration of the RNA was measured using a nanodrop spectrometer (Nanodrop® Technologies).

4.2.3 DNase digestion of RNA sample

To 100 µg of RNA (88 µl) 2 µl of DNase (Ambion) and 10 µl of 10 times reaction buffer was added. The RNA was incubated at 37°C in a water bath for 30 min.

4.2.4 RNA purification using RNeasy® MinElute Cleanup kit (QIAGEN®)

The RNA was purified after DNase digestion using the RNeasy ® MinElute Cleanup kit (QIAGEN®) as per manufacturer's instructions. To the 1.5 ml tube containing the RNA following DNase digestion, 350 µl of Buffer RLT was added and the tube was well mixed. Pure ethanol (200 µl) was added to the 1.5 ml tube and mixed using a pipette. The sample was transferred to a RNeasy MinElute spin column, which had been placed in a 2 ml collection tube. The column was centrifuged at 8,000 x *g* for 15 sec and the flow-through was discarded. The spin column was placed in a fresh collection tube and 500 µl of Buffer RPE was added. The column was centrifuged at 8,000 x *g* for 15 sec and the flow through was discarded. 80 % ethanol (500 µl) was added to the spin column and it was centrifuged at 8,000 x *g* for 2 min. The flow-through and collection tube were discarded. The column was placed in a fresh collection tube and centrifuged at 14,200 x g for 5 min. The flow-through and collection tube were discarded. The spin column was placed in a fresh 1.5 ml tube and 14 µl of RNase free water was added to the spin column. The column was centrifuged at 14,200 x *g* for 1 min to elute the RNA.

4.2.5 cDNA library construction from total RNA

The cDNA library was then synthesised from the RNA using the SMART cDNA library construction kit (Clontech). To a 0.5 ml tube, 0.4 µl of RNA (1µg of RNA), 2.6 µl of RNase free water, 1 µl of SMART VI Oligonucleotide and 1 µl of CDS III/3' PCR primer were added. This was centrifuged briefly and incubated at 72°C for 2 min, then placed straight onto ice for a further 2 min. The tube was centrifuged briefly to collect the content at the bottom. Then to the same tube, 2 µl 5 x First-strand Buffer, 1 µl DTT (20 mM), 1 µl dNTP mix (10 mM) and 1 µl PowerScript Reverse Transcriptase were added. The sample was incubated at 42°C for 1 hr in an air incubator and then put on ice to stop the 1st strand synthesis.

The 2nd cDNA strand was synthesised by PCR. The GeneAmp 2400 PCR machine was preheated to 95°C. To a 0.5 ml PCR tube, 2 µl First strand cDNA, 80 µl dH_2O, 10 µl 10x Advantage 2 PCR buffer, 2 µl 50x dNTP mix at 10 mM per dNTP, 2 µl 5' PCR Primer, 2 µl CDS III/3' PCR Primer and 2 µl 50x Advantage 2 Polymerase mix were added. The program 1 cycle at 95°C for 20 sec, then 20 cycles of 95°C for 5 sec and 68°C for 6 min was run on the preheated PCR machine. A 5 µl aliquot was examined by electrophoresis on a 1.1 % agarose gel, alongside a 1 kb DNA ladder. cDNA appeared as a smear between 0.1 and 4 kb.

The QIAquick PCR purification kit (QIAGEN®) was used to purify the cDNA sample (protocol as described in 2.2.13).

The cDNA was size-fractionated using CHROMA SPIN-400 (Clontech) size exclusion columns. At RT, a spin column matrix was re-suspended using a 1 ml pipette. The bottom cap was removed and the buffer was allowed to drain. Carefully, 700 µl of column buffer was added to the top of the column. cDNA (50 µl) was added to the top of column matrix. To the tube, 100 µl of column buffer was added to rinse any residual cDNA, which was then added to the column. The buffer and cDNA was allowed to absorb into the matrix. To the column, 600 µl of column buffer was added and the drops were collected one per tube.

A 1.1 % agarose gel was prepared as described in 2.2.9 (using 0.66 g of agarose) and 3 µl of each drop with 0.5 µl of loading buffer was examined by electrophoresis. The four fractions that had a smear of cDNA between 0.5 and 2 kb were collected to give a total volume of 125 µl.

The cDNA was precipitated using reagents supplied in the SMART cDNA synthesis kit (Clontech). To the pooled cDNA 0.1 volumes of sodium acetate (3 M; pH 4.8), 1.3 µl of glycogen (20 mg/ml) and 2.5 volumes of 95 % ethanol (-20°C) were added. The tube was placed on an orbital rocker to gently mix, and then incubated at -20°C for 1 hr.

The tube was centrifuged at 14,200 x *g* for 20 min to pellet the cDNA. The supernatant was carefully removed and discarded. The tube was centrifuged again briefly at 14,200 x *g* and the last drop of fluid removed. The pellet was air dried for 10 min then 7 µl of dH_2O was added. The concentration of the cDNA was measured using a nanodrop spectrometer.

4.2.6 Suppression subtractive hybridization

A cDNA expression library of feeding-induced genes was prepared by Dr. A. Nisbet (Moredun Research Institute) using the PCR Select synthesis kit (Clontech) (Diatchenko et al., 1996). cDNA was synthesised from mRNA extracted from fed or starved mites, as described in 4.2.1.

SSH was performed according to the manufacturer's protocol (PCR-Select™ cDNA subtraction kit, Clontech) with minor modification (see Nisbet et al., 2008). This procedure involved the synthesis of cDNA from fed or starved mite mRNA by reverse transcription, digestion of the cDNA with endonuclease *Rsa*I and ligation of adapters to the digested fragments to produce 'tester' cDNA for fed mites. The "fed tester cDNA" was then hybridised with an excess of starved cDNA ('driver') to remove common cDNA species from the adapter-ligated, fed cDNA. Hybridised

fractions were then subjected to PCR to amplify differentially expressed cDNAs. PCR-amplified, fed-specific cDNA, produced by SSH, was ligated into the pGEM®-T Easy vector (Promega) and transformed into competent *Escherichia coli* (strain JM109, Promega). Individual positive clones were picked (based on blue/white selection), and the plasmid was purified and sequenced.

Semi-quantitative real-time PCR (RT-PCR) was used to verify the differential expression in genes expressed in the SSH library. Single stranded cDNA (ss-cDNA) was synthesized from total RNA isolated from fed or starved mites using oligo dT primer and the Superscript II kit (Invitrogen) according to the manufacturer's instructions. These ss-cDNAs were used as templates in semi-quantitative PCR reactions using internal primers specific for each of six randomly selected ESTs identified during initial sequencing of the "fed-specific" library. The following cycling conditions were used: 94°C for 5 min followed by up to 28 cycles of 94°C for 15 sec, 53°C for 30 sec, 72°C for 30 sec with a final 10 min extension at 72°C. Negative controls were performed where ss-cDNA was replaced by 1 µg of total RNA. No amplification of products from contaminating genomic DNA was seen. Amplification products were separated on a 1.5 % agarose gel, prepared as described in 2.2.9 (using 0.9 g of agarose).

4.2.7 Sequencing of Expressed Sequence Tags

cDNA from the SSH (4.3.4) or unsubtracted library (4.3.2) was used to transform *E. coli* JM109 competent cells as described in 2.2.10. Colonies that were non-contiguous were picked from the plates.

LBG broth (150 µl) was added to each well of five 96-well plates. Pipette tips were used to pick colonies from the plates and add the cells to the wells. The tips were left in the wells for at least 10 min before being discarded. The plates were incubated overnight at 37°C. These plates were termed the Master plates.

To another 96 well plate, the Working plate, 50 µl of cell suspension was transferred from each well of the Master plate. A PCR was set up for one of the plates to make sure the plasmid had an insert, i.e. to establish the transformation rate. A Master mix was prepared using 200 µl of M13 Forward primer (10 pmol/µl), 200 µl of M13 Reverse primer (10 pmol/µl), 200 µl of 10 x Buffer(1.5 mM $MgCl_2$), 1300 µl of H_2O, 20 µl of Taq polymerase (5 U/µl) and 40 µl of dNTPs (2 mM) . A multi-channel pipette was used to dispense 18 µl into each well of a 96-well PCR plate. Cell suspension (2 µl) was pipetted from the Working plate to the PCR plate as a template. The plates were foil heat sealed to prevent evaporation. A 1 % agarose gel was poured with six rows of 18 wells to make sure the PCRs were successful before sequencing. Ethidium bromide (1.5 µl) was used as a dye.

The PCR products were purified using the restriction Exonuclease 1 and Shrimp Alkaline Phosphotase (SAP), as described in 2.2.14. The plates were then set up for sequencing. A sequencing Master mix was prepared using 200 µl of 5x Sequencing Buffer, 434 µl of ddH_2O, 150 µl of BigDye 3.1 mix and 16 µl of Primer (1.6 pmol per reaction) per plate. The plates were put on a FAST PCR machine using the folowing program; one cycle for 96°C for 1 min, 25 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 75 sec. The plates were stored at 4°C until required. The plates were sequenced on the 3730 DNA Analyzer.

The EST sequences were organised into clusters, and homology searches were performed using the programs Trace2BDEST and PartiGene (Parkinson et al., 2004) to search databases uniprot, arthclus and rRNAe. The sequences were put into a database, which was mounted on the web (http://xyala.cap.ed.ac.uk/NeglectedGenomes/Dermanyssus/).

4.2.8 Roche 454 sequncing

RNA was extracted, DNase-treated and purified from freshly collected *D. gallinae*, as described in 4.2.2-4.2.4. An aliquot of this RNA (10 µg) was used for Roche 454

sequencing by The GenePool Sequencing Service following the manufacturer's instructions (Margulies et al., 2005).

4.2.9 Illumina Solexa sequencing

RNA was extracted from three populations of freshly collected *D. gallinae*, approximately 60 % of which were engorged, and from starved mites as described in 4.2.2. The RNA was DNase digested, as described in 4.2.3, and then purified, as described in 4.2.4. RNA (30 µg) from three samples of fed mites and three samples of starved mites, which were biological replicates, was sent to the GenePool Sequencing Service for Solexa sequencing, where the manufacturer's protocol was followed (Morrissy et al., 2009).

The data received from the Illumina Solexa sequencing was BLAST searched against the database generated from the Roche 454 sequencing described in 4.3.3 to identify differentially expressed transcripts from fed and starved mites.

The GeneSpring GX 11.0 software was used to analyse the data. Only the sequences that had homology to contigs from the Roche 454 database and were identified in all six samples were used in the analysis. A filter was applied that removed all the contigs that had less than a threefold change between the fed and starved groups, either up- or down-regulated. An unpaired Benjamini and Hochberg t-test was performed on each of the contigs with and without multiple test correction (MTC).

4.3 Results

4.3.1 Expressed sequence tag (EST) database generated from a *D. gallinae* cDNA library

An EST database was generated, as described in 4.2.7, from a library constructed from cDNA, as described in 4.2.5.

The database was generated from 398 sequences and these produced 252 ESTs formed from the individual sequences. Fifty of these were contigs generated from multiple sequences and the other 202 ESTs were individual sequences. BLAST analysis was carried out, first translating the nucleotide sequence (blastx) then comparing the amino acid sequence to the NCBI non-redundant (nr) protein database. Secondly, the nucleotide sequences (blastn) were compared to a database of all eukaryote ribosomal RNA (rRNA) sequences (The GenePool, Edinburgh University). Homologous sequences to 42 of the 252 contigs were identified. The other 210 contigs had no significant hit in either of the databases. Table 4.1 contains all the contigs with significant hits ($p \leq 0.05$).

Table 4.1. List of the contigs with significant hits against either the NCBI nr database or the eukaryote rRNA database kept at The GenePool (Edinburgh University). The "Nos. of EST" was the number of individual sequences used to form each contig. The column labelled "Length" was the length of the contig in bases. The column marked "Top Hit" has a description of the top hit from the BLAST analysis, except where noted in the description. The Database column showed which database the homologous protein was found in.

Contig	Nos. of EST	Length	Top Hit	Database	Score	e value
DCG00056	17	1080	5.8S ribosomal RNA gene AF061796 [*Branchiostoma floridae*]	rRNAe	551	1E-157
DCG00005	33	2279	Non-structural polyprotein, Kashmir bee virus	NCBI nr	320	1E-139
DCG00031	1	668	Polyubiquitin [*Tribolium castaneum*] (Not top Hit)	NCBI nr	262	2E-70
DCG00226	1	732	Cytochrome oxidase subunit I CAP58872.1 [*Dermanyssus gallinae*]	NCBI nr	254	6E-66
DCG00181	2	296	18 rRNA gene X77784 [*X.vesparum*]	rRNAe	246	2E-65
DCG00026	1	126	18S ribosomal RNA gene L76343 [*Cosmolaelaps trifidus*]	rRNAe	234	2E-62
DCG00222	1	798	Alpha-parvin, putative [*Ixodes scapularis*]	NCBI nr	197	1E-48
DCG00166	1	746	Cuticular protein putative [*Ixodes scapularis*]	NCBI nr	191	9E-47
DCG00209	1	771	Hypothetical protein BRAFLDRAFT_255626 [*Branchiostoma floridae*]	NCBI nr	178	5E-43
DCG00102	1	668	Similar to GA19430-PA [*Nasonia vitripennis*]	NCBI nr	140	1E-31
DCG00079	1	690	39S ribosomal protein L51 [*Rhipicephalus sanguineus*]	NCBI nr	140	2E-31
DCG00094	1	720	Arginyl-tRNA synthetase [*Xenopus (Silurana) tropicalis*]	NCBI nr	139	4E-31
DCG00159	1	751	Similar to RIKEN cDNA 1810011O16 isoform 6 [*Canis familiaris*]	NCBI nr	135	6E-30
DCG00269	1	637	Translation elongation factor 2 [*Lycosa singoriensis*]	NCBI nr	127	8E-28
DCG00130	2	801	Ly1 antibody reactive homolog [*Saccoglossus kowalevskii*]	NCBI nr	124	9E-27
DCG00082	2	663	Lysine-specific demethylase NO66 [*Bos taurus*]	NCBI nr	122	3E-26
DCG00034	2	557	Proteasome maturation protein (Proteassemblin) [*Homo sapiens*]	NCBI nr	120	9E-26
DCG00040	1	571	Similar to TRAP/Mediator complex component TRAP25 isoform 1 [*Apis mellifera*]	NCBI nr	105	3E-21
DCG00027	6	492	16S ribosomal RNA gene AF177143 [*Pseudhemilea longistigma*]	rRNAe	100	4E-21
DCG00086	1	316	Aminoimidazole-4-carboxamide ribonucleotidetransformylase/IMP cyclohydrolase [*Ixodes scapularis*]	NCBI nr	102	1E-20

Contig	Nos. of EST	Length	Top Hit	Database	Score	e value
DCG00193	1	662	Secreted protein, putative XP_002433530.1 [*Ixodes scapularis*]	NCBI nr	103	1E-20
DCG00098	1	266	Ribosomal protein S15A [*Lysiphlebus testaceipes*]	NCBI nr	99	2E-19
DCG00038	1	531	Replication-associated protein [*Gull circovirus*]	NCBI nr	94	8E-18
DCG00259	1	485	rad25/xp-B DNA repair helicase, putative XP_002399857.1 [*Ixodes scapularis*] (Second Hit)	NCBI nr	91	4E-17
DCG00215	3	606	40S ribosomal protein S13 [*Ixodes scapularis*]	NCBI nr	90	1E-16
DCG00063	2	578	10 kDa putative secreted protein [*Argas monolakensis*]	NCBI nr	109	3E-16
DCG00019	3	582	Cytoplasmic cystatin [*Ixodes scapularis*]	NCBI nr	88	6E-16
DCG00157	1	431	26S protease regulatory subunit S10B [*Anoplopoma fimbria*] (Second Hit)	NCBI nr	76	9E-15
DCG00163	1	736	Venom serine protease precursor [*Polistes dominulus*]	NCBI nr	82	6E-14
DCG00066	2	177	Endomyces fibuliger 8014 17S ribosomal RNA and 5.8S ribosomal RNA genes, complete sequence	rRNAe	70	1E-12
DCG00171	1	466	Hypothetical protein IscW_ISCW007013 XP_002408298.1 [*Ixodes scapularis*]	NCBI nr	69	2E-10
DCG00141	2	360	Glutathione S-transferase putative [*Ixodes scapularis*]	NCBI nr	68	4E-10
DCG00060	1	566	KNSL2 protein [*Oryzias latipes*]	NCBI nr	66	2E-09
DCG00043	1	732	Nonstructural polyprotein [*Solenopsis invicta*] virus 1	NCBI nr	65	5E-09
DCG00018	41	288	25S ribosomal RNA gene M26190 [*M. racemosus*]	rRNAe	52	5E-07
DCG00198	1	544	Defender against apoptotic cell death 1 [*Araneus ventricosus*]	NCBI nr	57	7E-07
DCG00172	1	644	Conserved hypothetical protein XP_002434012 [*Ixodes scapularis*]	NCBI nr	52	3E-05
DCG00107	1	256	Beta-ureidopropionase, putative [*Ricinus communis*] (Not top hit)	NCBI nr	49	1E-04
DCG00096	1	192	DNA-directed RNA polymerase [*Cytophaga hutchinsonii*]	NCBI nr	46	1E-03
DCG00148	1	629	Similar to GTP binding protein 3 [*Ciona intestinalis*]	NCBI nr	45	5E-03
DCG00131	1	353	NADH:ubiquinone oxidoreductase, NDUFB6/B17 subunit, putative [*Ixodes scapularis*]	NCBI nr	43	1E-02
DCG00184	1	254	Ribosomal RNA (rRNA) gene L78065 [*Anopheles albimanus*]	rRNAe	68	7E-01

In the database there were ten contigs with significant homology to rRNA molecules, from both the NCBI nr and the eukaryote rRNA databases. The contig which was made up of the highest number of individual sequences (41) was homologous to a ribosomal RNA (rRNA) molecule. Of the 42 contigs that had significant homology to other proteins, 11 had homology to proteins expressed in ticks of the *Ixodes* genus. Nine of the contigs were homologous to enzymes, one of which has been identified as cytochrome oxidase subunit 1 from *D. gallinae* (Roy et al., 2009) and another was homologous to the deer tick *Ixodes scapularis* glutathione S-transferase (GST). The Kashmir bee virus was represented by a large number of individual sequences (33) that clustered together into a single contig 2279 bases long. Another two of the contigs were homologous to other viral proteins.

4.3.2 EST database generated from an SSH cDNA library

An EST database was generated using an SSH library constructed from cDNA made by suppression subtractive hybridization, as described in 4.2.6.

Semi-quantitative RT-PCR, as described in 4.2.6, was used to verify the differential expression in genes expressed in the SSH library. Figure 4.1 is a photograph of a gel on which the PCR products were examined. Three of the gene expression levels were higher in the fed sample than the starved sample. The control samples were a similar intensity for both samples.

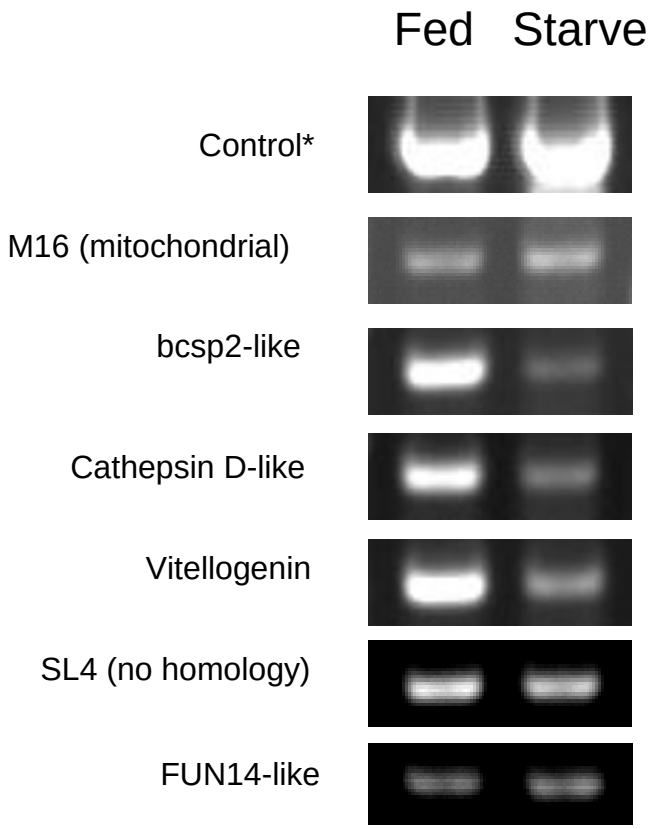


Figure 4.1. Semi-quantitative RT-PCR to confirm differential expression of transcripts represented in fed *D. gallinae*. * Control shows ribosomal band from equal quantities of RNA from fed and starved mites used in the cDNA synthesis step of the RT-PCR. Reproduced with consent of Dr. A. Nisbet.

Individual clones from the library were grown in bacterial colonies on agar plates. Colonies (960) were picked and sequenced, as described 4.2.7. Of the 960 sequencing reactions, 770 had good traces and were used to form 310 contigs. After BLAST analysis comparing the contigs using blastx, to the NCBI nr database and then KEGG database 133 of the contigs had significant homology to proteins in the database ($e \leq 0.05$).

The contigs which had significant homology to a protein in either database were grouped together based on their function. Figure 4.2 is a pie chart that shows the proportion of individual sequences, which clustered to form the contigs in each group, of the total number of individual sequences in the database. A table of all the contig descriptions along with the length of the contig, the number of individual sequences that formed the contig, the *p* value, the species from which the

homologous protein was characterised and the database containing the homologous protein was tabulated and shown in Appendix 1. InterProScan (Hunter et al., 2009; Quevillon et al., 2005) was used to identify regions of the contigs that were conserved protein domains.

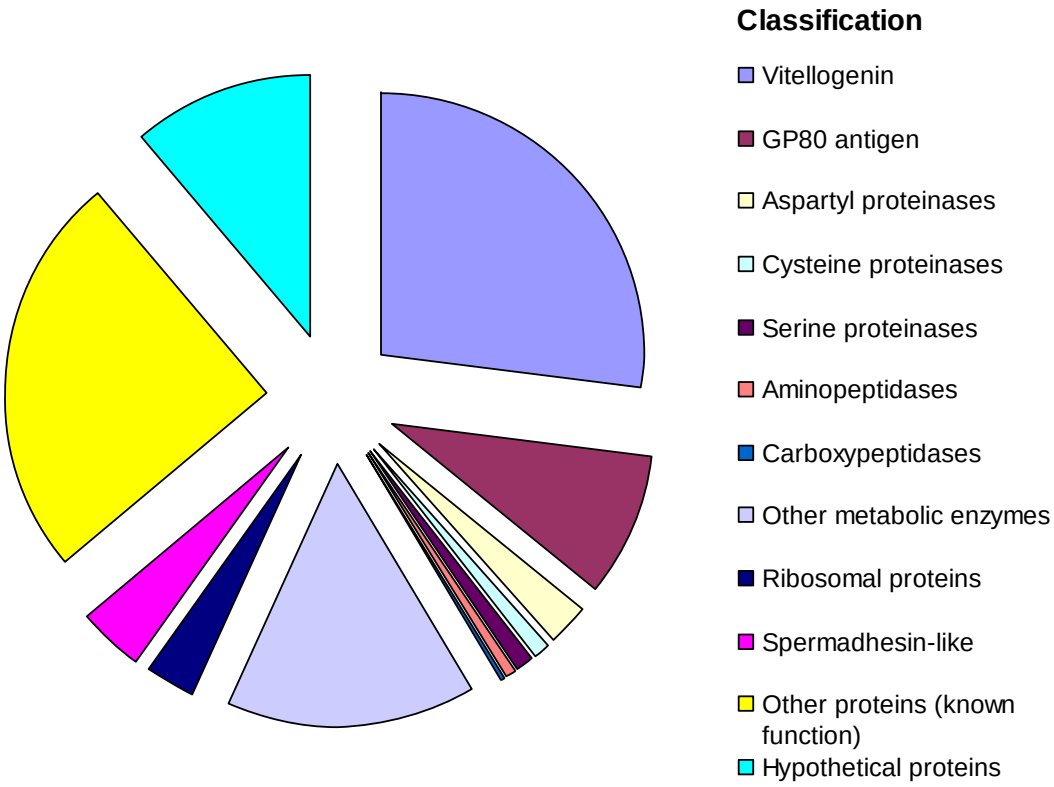


Figure 4.2. Pie chart showing proportion of the number of sequences used to build contigs with known homologue to the total number of good sequences used to make the SSH EST database. The number of sequences used to build each contig within a classification was calculated as a proportion of the total number of readable sequences.

The largest proportion of individual sequences differentially expressed in the fed group (9.2 %) formed contigs that were homologous to vitellogenin. The second highest proportion of individual sequences that made up contigs homologous to a single protein was 2.9 %, the protein being GP80, a derivative of vitellogenin. Proteinases, peptidases and other metabolic enzymes were highly represented in the fed library with 7.1 % of the total number of individual sequences. Most notably,

subtraction of fed versus starved mites removed the predominance of ribosomal RNA from the derived sequences observed in the initial EST database described in 4.3.1.

4.3.3 Roche 454 database

From the Roche 454 sequencing reaction, 4,014,119 bases of sequence were produced, which formed 13,363 contigs, the longest of which was 8,641 bases. The contigs were compared to NCBI nr protein database, using the blastx function. From this search 3,418 (26 %) of the contigs had a significant hit to a protein of known function. 6,114 (46 %) of the contigs had no homology to any protein in the database. The rest of the sequences had a positive hit to a protein but an e-value greater than 0.05, which was considered non-significant.

The NCBI clusters of orthologous groups (COGs) of proteins function was used to identify the functional classification of the 13,363 contigs built from the Roche 454 sequencing. The contigs that did not match any protein in the database or those with no COG reference (9201 contigs; 69 %) were removed from the annotated dataset. The contigs that had homologues (4162 contigs, 31 %) were allocated to one or more of four main classifications, as described on the COG website; “Information Storage and Processing (I)”, “Cellular Processes and Signalling (C)”, “Metabolism (M)” and “Poorly Characterised”. The proteins that were classified as “Poorly Characterised” only were removed, since the proteins were of unknown function or the function was generalised. Of the 4162 contigs with homologues in the databases 852 contigs were “poorly classified”. Figure 4.3 is a pie chart showing the proportion of contigs in each of the remaining classifications (I, C or M). Tables showing the contig number, a brief description of the top hit from the NCBI nr database, e value, and the classifications are shown in Appendix 2.

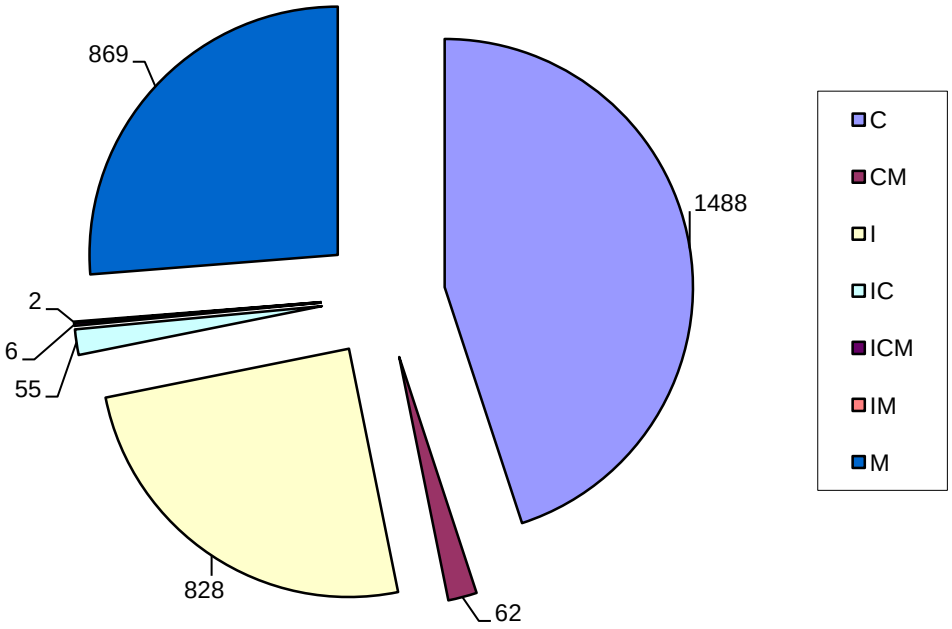


Figure 4.3. Pie chart showing the proportion of contigs from the Roche 454 sequencing in each of the major classifications. The single letter codes in the legend were: C, Cellular Processes and Signalling; I, Information Storage and Processing and M, Metabolism. The numbers on the pie chart were the number of contigs in each classification (3310 contigs in total).

The contigs in the "Cellular Processes and Signalling" group, which accounted for 45 % of all the total significant hits, were split into 10 sub-categories. The sub-category with the largest percentage of contigs (32 %) was Post-translational modification, protein turnover, chaperones (o), which comprised 481 contigs. This group included proteins such as cathepsins L, Z and D, (Dalton et al., 2003; Lustigman et al., 2004; Verity et al., 2001) and glutathione S-transferases (Sexton et al., 1990), which have been shown to be possible vaccine candidates in other blood-feeding parasitic species.

The contigs that were classified in the "Metabolism" categories comprised 26 % (869 contigs) of the total contigs classified by COG. These contigs were sub-classified into eight catagories. There were 183 contigs (21 %) that had homology to proteins involved in Energy production and conversion (c).

Of the 828 contigs that form the "Information storage and processing" classification, 25 % of the total number of contigs that had COG classifications, 36 % were sub-classified as Translational, ribosomal structure and biogenesis (j). Many of the contigs in this sub-classification were ribosomal proteins. Another 20 % of the contigs had homology to proteins involved in protein transcription (k).

The remaining 4 % of contigs (125) were allocated into at least two of the above categories.

4.3.4 Illumina Solexa database

The data from the Illumina Solexa sequencing was filtered so that only contigs that were identified in all six samples and that had homology to contigs from the Roche 454 database were used in the analysis (2086 contigs). A second filter was applied that removed all the contigs that were not differentially expressed by at least three-fold between the fed and starved groups, either up- or down-regulated (448 contigs). There were 66 contigs and 227 contigs that were significantly differentially expressed between the fed and starved RNA samples when the test was performed with and without multiple test correction, respectively ($p \leq 0.05$).

Table 4.2 contains the description of the 66 contigs, which were up-regulated in fed mites, along with the contig number from the Roche 454 database, the fold change, corrected *p* value, *p* value, a description of the contig and the number of sequences counted in each sample. The top two contigs with the highest fold change were up-regulated and homologous to vitellogenin. Other transcripts that were up-regulated encoded a cathepsin L homologue and various salivary proteins. Four transcripts were down-regulated, one of which encoded a protein which was homologous to the myosin heavy chain. The contigs that were not significantly differentially expressed when the multiple test correction was used during the t–test were listed in Appendix 3 in the same format as table 4.2. This list included the 66 contigs that were shown in table 4.2 (227 contigs).

Figure 4.4 is a graph that shows the distribution of the fold change of the 66 contigs. The majority of the contigs were up-regulated in fed mites. Of those contigs, more than half (36 contigs) had a fold change of greater than three and less than or equal to six.

Table 4.2. Table showing all the contigs that had count data in each sample and had a significant fold change of more than three with multiple test correction. The column marked Probe contains Roche 454 reference of the sequence. The fold change was calculated such that a positive value represents an up-regulated protein and a negative was down-regulated. The corrected *p* value was generated using t-test with MTC and the *p* value was generated without MTC. The gene description was taken from the Roche 454 database. Columns F1, F2, F3, S1, S2 and S3 contained the number of sequences from each RNA sample (F = Fed sample, S = Starved sample).

Probe	Fold change Fed vs Starved	Corrected *p* value	*p* value	Gene description	F1	F2	F3	S1	S2	S3
contig12543	110.853	0.016	0.00026	gi|17570195|ref|NP_508868.1| VITellogenin structural genes (yolk... 60	28738	99606	52525	561	361	545
contig12032	69.317	0.042	0.00511	gi|82878755|gb|AAW78557.2| vitellogenin [*Dermacentor variabilis*] 209	7916	2465	2294	150	16	56
contig12020	58.038	0.044	0.00587	No hits found	11528	3657	8541	473	66	59
contig12579	55.864	0.009	6.5E-05	gi|169215918|ref|XP_001718712.1| PREDICTED: hypothetical protein... 42	11859	7087	10710	191	124	218
contig00712	39.675	0.011	0.00012	gi|167035216|ref|YP_001670447.1| hypothetical protein PputGB1_42... 35	9327	11563	15466	225	271	438
contig00149	38.910	0.008	1.7E-05	No hits found	6404	7744	8025	224	145	208
contig12724	36.924	0.030	0.00204	gi|74267388|dbj|BAE44191.1| cuticular protein [*Tachypleus triden*... 50	1071	1231	2291	25	25	96
contig01395	35.517	0.022	0.00082	gi|119715610|ref|YP_922575.1| hypothetical protein Noca_1374 [No... 35	1471	1353	1569	24	88	33
contig12013	26.812	0.034	0.00267	gi|82878755|gb|AAW78557.2| vitellogenin [*Dermacentor variabilis*] 831	127950	71499	50643	5890	1400	2915
contig12861	21.875	0.025	0.00126	gi|158316180|ref|YP_001508688.1| hypothetical protein Franean1_4... 35	648	1276	1940	43	44	81
contig12148	21.540	0.011	0.00015	gi|170696288|ref|ZP_02887419.1| transcriptional regulator, winge... 34	2905	1962	3000	85	136	148
contig04772	17.297	0.042	0.00521	No hits found	1530	1486	4942	85	103	248
contig00317	13.486	0.041	0.00433	No hits found	369	532	1417	45	36	70
contig00690	12.945	0.022	0.00092	gi|56684769|gb|AAW22505.1| ferritin heavy chain-1b [*Carcinoscorp*... 160	462	270	378	19	44	26
contig13306	11.777	0.022	0.00098	gi|195114014|ref|XP_002001562.1| GI16461 [*Drosophila mojavensis*]... 275	2917	2853	4239	341	168	377
contig12606	10.089	0.042	0.00500	gi|109506318|ref|XP_226158.4| PREDICTED: similar to Mucin-2 prec... 57	2532	753	2552	158	153	196

Probe	Fold change Fed vs Starved	Corrected *p* value	*p* value	Gene description	F1	F2	F3	S1	S2	S3
contig12810	9.969	0.030	0.00184	gi|114675653|ref|XP_001170388.1| PREDICTED: deoxyribonuclease II... 119	1910	833	1827	172	104	164
contig00670	9.900	0.025	0.00127	gi|72008176|ref|XP_780713.1| PREDICTED: similar to cathepsin L [... 377	3956	4789	8377	741	541	408
contig13131	9.699	0.041	0.00447	gi|91081217|ref|XP_975622.1| PREDICTED: similar to ecdysteroid r... 116	823	1202	2413	89	200	147
contig11417	8.536	0.016	0.00041	gi|91080885|ref|XP_972748.1| PREDICTED: similar to GA14321-PA [T... 37	97	73	137	12	10	13
contig11787	8.513	0.016	0.00035	gi|15219757|ref|NP_176856.1| unknown protein [*Arabidopsis thalia*... 42	74679	140492	100406	12198	12937	10821
contig11836	7.939	0.030	0.00187	gi|40548304|ref|NP_954972.1| carboxypeptidase, vitellogenic-like... 303	4773	1846	2783	321	376	406
contig12768	7.628	0.020	0.00065	gi|196236958|ref|ZP_03135749.1| conserved hypothetical protein [... 49	4136	3918	7235	698	550	688
contig12017	7.522	0.043	0.00561	No hits found	2824	1319	2093	350	145	361
contig13173	7.215	0.009	6.3E-05	gi|1389604|gb|AAC59905.1| acetylcholinesterase 186	13321	12603	16570	2172	2027	1682
contig11590	6.225	0.020	0.00057	gi|149287022|gb|ABR23410.1| p8 nuclear protein [*Ornithodoros par*... 80	7885	6337	6074	776	1263	1284
contig13129	5.688	0.047	0.00650	gi|118429511|gb|ABK91803.1| aspartic protease precursor [*Clonorc*... 177	1625	666	1574	226	266	154
contig11293	5.372	0.047	0.00662	gi|148717315|dbj|BAF63671.1| protein disulfide isomerase-2 [*Haem*... 385	1079	991	1433	118	261	321
contig12246	5.285	0.025	0.00141	gi|194226870|ref|XP_001917242.1| PREDICTED: similar to adenylosu... 263	800	656	796	102	136	204
contig13068	4.948	0.035	0.00303	gi|27733415|gb|AAO21505.1|AF413064_1 serpin 3a [*Manduca sexta*] >... 35	86	141	113	26	29	15
contig01538	4.922	0.047	0.00647	gi|47522760|ref|NP_999131.1| long-chain 3-ketoacyl-CoA thiolase ... 129	236	261	262	36	94	40
contig01772	4.839	0.042	0.00507	gi|169217278|ref|XP_001732938.1| PREDICTED: hypothetical protein... 34	1855	924	873	316	209	200
contig12894	4.807	0.041	0.00395	gi|114153234|gb|ABI52783.1| mucin peritrophin salivary protein [... 70	10902	25028	18318	3651	2948	4181
contig02157	4.781	0.020	0.00072	gi|67083785|gb|AAY66827.1| secreted salivary protein [*Ixodes sca*... 48	2033	2647	2771	406	517	650
contig11681	4.723	0.025	0.00141	No hits found	270	184	294	55	63	40
contig07116	4.637	0.041	0.00447	No hits found	318	384	429	54	128	76
contig02324	4.524	0.041	0.00413	gi|41617918|tpg|DAA02898.1| TPA: TPA_inf: HDC09484 [*Drosophila m*... 33	236	544	385	70	93	82
contig11943	4.511	0.041	0.00374	gi|171912587|ref|ZP_02928057.1| hypothetical	752	516	833	102	194	178

Probe	Fold change Fed vs Starved	Corrected *p* value	*p* value	Gene description	F1	F2	F3	S1	S2	S3
				protein VspiD_15445... 33						
contig03269	4.501	0.030	0.00197	No hits found	2036	3587	2760	784	530	532
contig00212	4.364	0.043	0.00561	gi|193594270|ref|XP_001942800.1| PREDICTED: similar to S-adenosy... 154	357	180	268	85	46	53
contig00498	4.352	0.034	0.00261	gi|88602953|ref|YP_503131.1| HNH endonuclease [*Methanospirillum* ... 36	4921	2929	3131	793	1072	644
contig07176	3.996	0.041	0.00381	gi|28422163|gb|AAH46839.1| Plk-prov protein [*Xenopus laevis*] 147	885	1163	1322	209	405	252
contig10262	3.991	0.034	0.00272	gi|74318688|ref|YP_316428.1| metal dependent phosphohydrolase, H... 36	176	117	129	25	44	38
contig13261	3.972	0.016	0.00034	gi|157776976|ref|XP_001666554.1| hypothetical protein CBG15153 [... 65	630	597	700	181	180	129
contig07208	3.846	0.041	0.00411	gi|118462588|ref|YP_882079.1| CDP-alcohol phosphatidyltransferas... 37	499	331	460	84	100	159
contig11796	3.842	0.041	0.00432	gi|82705844|ref|XP_727137.1| hypothetical protein PY06477 [*Plasm*... 39	1376	1865	1151	278	359	522
contig04085	3.688	0.016	0.00042	gi|170094979|ref|XP_001878710.1| predicted protein [*Laccaria bic*... 33	421	449	326	97	123	103
contig03700	3.684	0.041	0.00426	gi|191171858|ref|ZP_03033404.1| Cps2D [*Escherichia coli* F11] >gn... 33	767	464	416	141	178	118
contig11620	3.591	0.047	0.00665	gi|149287114|gb|ABR23456.1| cytochrome c oxidase polyprotein IV ... 50	237	202	392	65	99	63
contig12001	3.529	0.049	0.00722	gi|50758110|ref|XP_415765.1| PREDICTED: similar to Malate dehydr... 326	769	1149	1243	436	246	233
contig09563	3.514	0.041	0.00459	gi|169213190|ref|XP_001714612.1| PREDICTED: hypothetical protein... 34	74	48	61	24	13	16
contig04310	3.443	0.025	0.00134	gi|158287073|ref|XP_309120.3| AGAP000935-PA [*Anopheles gambiae* s... 305	409	371	600	127	122	144
contig12071	3.434	0.034	0.00253	gi|109118856|ref|XP_001109939.1| PREDICTED: acid alpha-glucosida... 61	1354	1749	1891	370	628	476
contig00470	3.313	0.009	7.9E-05	gi|156353921|ref|XP_001623156.1| predicted protein [*Nematostella*... 66	5270	5577	4710	1466	1497	1735
contig11441	3.293	0.042	0.00473	gi|148657070|ref|YP_001277275.1| putative endoribonuclease L-PSP... 143	1935	3275	3082	647	1005	841
contig00543	3.287	0.049	0.00725	gi|170596615|ref|XP_001902831.1| FKBP-type peptidyl-prolyl cis-t... 103	1640	1074	1284	276	418	552
contig00223	3.269	0.022	0.00091	No hits found	3822	3836	5632	1437	1264	1301
contig01770	3.234	0.029	0.00166	gi|189240738|ref|XP_968166.2| PREDICTED: similar to AGAP007574-P... 107	586	461	712	209	152	179
contig00345	3.125	0.044	0.00595	No hits found	837	1438	1569	357	467	371

Probe	Fold change Fed vs Starved	Corrected *p* value	*p* value	Gene description	F1	F2	F3	S1	S2	S3
contig13047	3.100	0.042	0.00494	No hits found	3254	1913	1743	778	721	649
contig03384	3.089	0.016	0.00041	No hits found	178	212	200	69	53	70
contig12306	3.043	0.034	0.00259	gi|39653771|ref|NP_945222.1| NADH dehydrogenase subunit 5 [Haema... 125	30010	22941	36701	7970	10556	10662
contig00603	-4.429	0.035	0.00303	gi|77553801|gb|ABA96597.1| MYB10, putative, expressed [*Oryza sat*... 36	849	921	954	2547	4826	5273
contig07154	-4.765	0.035	0.00301	No hits found	36	49	42	193	138	301
contig04870	-5.137	0.032	0.00218	gi|183979376|dbj|BAG30740.1| muscle myosin heavy chain [*Papilio* ... 244	19	37	31	181	120	136
contig00606	-32.170	0.020	0.00071	gi|189242145|ref|XP_001813277.1| PREDICTED: similar to polyprote... 70	3212	1915	2612	149149	76820	46685

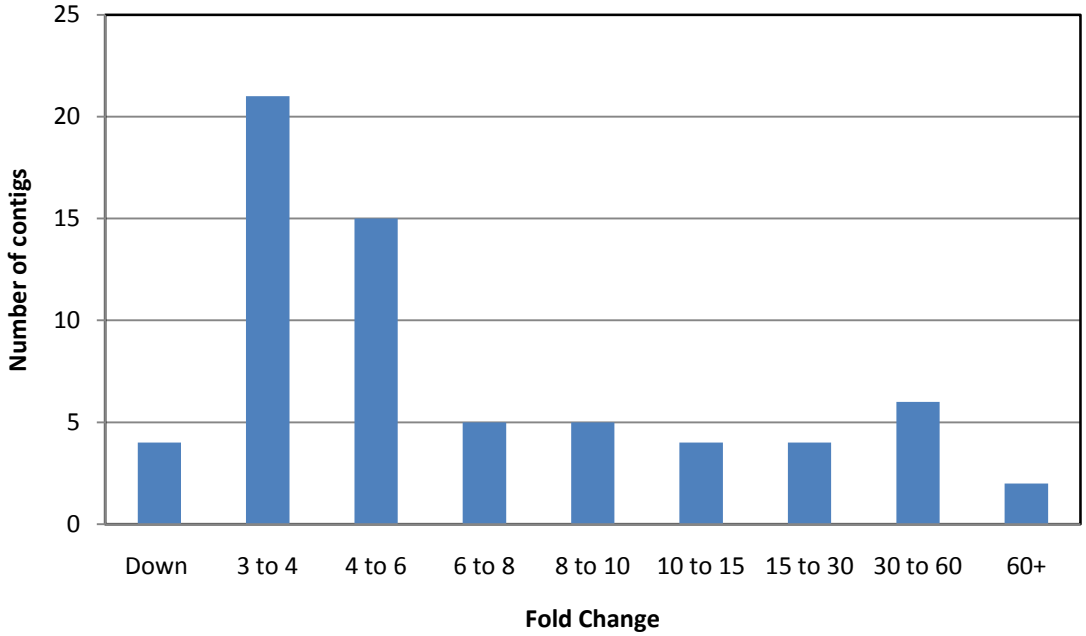


Figure 4.4. Frequency graph showing the number of contigs that had a fold change of over three between the number of sequences obtained from all three of the fed mite RNA samples and the number of sequences obtained from all three of the starved mite RNA samples. The values along the X-axis show fold changes between the fed and starved mites. The first column (Down) comprised of four contigs that were significantly down-regulated, with a fold change of more than three when the fed mites were compared to starved mites.

4.4 Discussion

The aim of the experiments described in this chapter was first to generate EST databases to provide EST sequences to identify homologues of possible vaccine candidates in *D. gallinae*; and second to use SSH and Illumina Solexa digital transcriptomics to identify transcripts that were differentially expressed between fed and starved populations of mites that could be potential vaccine candidates.

To generate the initial database, total RNA was extracted from a population of fed *D. gallinae* mites and converted into cDNA. The cDNA was then transformed into a bacterial system to grow colonies that were then sequenced using Sanger sequencing (Sanger et al., 1977) of individual clones. BLAST analysis was then performed to identify proteins homologous to previously characterised vaccine candidates in other parasitic species. This classical approach has been used to generate EST databases for other species of ectoparasite, for example *Psoroptes ovis* (Kenyon et al., 2003). Mining the EST database to identify possible vaccine candidates can be achieved by probing two dimensional Western blots, prepared using a crude mix of parasite protein, with sera from immunised animals. MALDI-ToF analysis of protein spots, detected by Western blot, would identify peptide fragments that can be identified in the EST database (Tawe et al., 2001).

The first database generated during the experiments described in this chapter contained only 46 contigs that had a significant homology to previously described sequences, 10 (22 %) of which were homologous to ribosomal sequences. These ribosomal RNA molecules were possibly present as an artefact of the synthesis step in the cDNA library construction, and demonstrates the problem of redundancy in this form of EST dataset mining.

In this initial EST database, nine of the contigs had significant homology to enzymes. Enzymes have been studied as potential vaccine candidates in many parasitic species, and interference with their function may be a factor in protection. Immunisation with *Leishmania* surface proteinase gp63 induces a protective immune response in mice

against a challenge of *Leishmania mexicana*. Mice that had been immunised with the recombinant protein and then challenged with 10^3 parasites found to have a total absence of infection 10 weeks after the challenge (Connell et al., 1993). The native H-gal-GP, a protein complex of *Haemonchus contortus* that contains a pepsin-like aspartyl protease and several metallopeptidases, generates an immunological response that protects against a challenge of *H. contortus*, reducing the worm burden by up to 83 % and egg production by up to 97 % (Smith et al., 2003).

One of the contigs was homologous to a glutathione S-transferase (GST) of *Ixodes scapularis*. GST is involved in the cellular detoxification process, and has been characterised and shown to reduce parasite burden by 78 % in GST-vaccinated sheep challenged with the liver fluke *Fasciola hepatica* (Rossjohn et al., 1997; Sexton et al., 1990). Another contig had significant homology to a serine protease precursor of the European paper wasp. The subsequent hits for this contig on the database were mostly serine proteases. On inspection of the BLAST alignments, a specific domain was recognised, and using InterProScan it was identified as the serine protease chymotrypsin. The proteinases hypodermin A, B and C, which are serine proteases, were purified from the instar stages of *Hypoderma lineatum*, a Dipteran which has a parasitic larval stage that lives in cattle, and were used to immunise naïve calves. The cattle develop a natural immunity to the parasitic stages after repeat infestation. Immunisation with these proteases generated an immune response in naïve animals comparable to the natural immunity during infestation. An antigen-specific lymphocyte response and antigen-specific antibody titres were observed in immunised animals. It was suggested that both the cellular and humoral pathways were involved in the immune response generated by the immunisation with these proteinases (Baron & Colwell, 1991). Serine proteases have also been used more recently to significantly lower parasite numbers when mice immunised with a serine protease were then challenged with $5x10^5$ *L. donovani* parasites, and showed complete protection. When the challenge was increased to $5x10^8$ parasites, an initial protection was observed but infection developed four months later (Choudhury et al., 2010).

Three of the contigs present were homologous to viral proteins. The longest contig in the database was homologous to the Kashmir Bee virus, which is a virus of the Family Dicistroviridae. It has a worldwide distribution and infects bee and wasp species (de Miranda et al., 2010). The virus present in the mites could be a relative of this virus, or the mites could be a reservoir, or both. The rest of the top 25 hits in the unipr database for this contig were all viruses, many of which were closely related to the Kashmir Bee virus.

The second EST database to be generated used Sanger sequencing technology to obtain the sequences of 960 cDNA clones from an SSH library constructed specifically to enrich for genes expressed after feeding. The common cDNA molecules between a library of fed and starved mites were removed, leaving only differentially expressed genes in fed mites. The contigs were organised into separate groups by hand since there were only 310 contigs in the database. After bioinformatic analysis, 133 contigs had significant homology to proteins in either the KEGG or the NCBI nr database, or had a protein domain predicted by InterProScan (Appendix 1).

The two contigs assembled from the highest number of individual sequences had homology to vitellogenin and GP80, a precursor of vitellogenin (Tellam et al., 2002). Of the next four contigs in the list, three were vitellogenin or contained vitellogenin domains, determined by InterProScan (Hunter et al., 2009). Vitellogenesis is the process of yolk formation, where nutrients are deposited in the oocyte. For ticks, a bloodmeal is required to initiate the process. The meal stimulates the production of vitellogenesis inducing factor, which in turn stimulates the tick to secrete a fat body stimulating factor that stimulates the fat body to produce vitellogenin (Ogihara et al., 2007). As previously described in 4.2.1, the mites collected were of mixed life stage and gender, and the majority were engorged. The female mites in the population would have been reproductively active and therefore ovigerous. Both purified native and recombinant GP80, from the tick *R. microplus*, have been used in a vaccine trial in cattle. The purified native protein reduced the numbers of engorged female ticks

by 25 %. However, the recombinant form of the protein had no effect on the ticks (Tellam et al., 2002).

As described previously, many different types of proteinases have been used to immunise hosts against a variety of parasites. Some of the contigs, comprising 7.2 % of the individual sequences, were homologous to proteolytic enzymes such cathepsin D, an aspartyl protease. Protection against the obligate haematophagous nematode *H. contortus* was generated after immunisation of sheep with a native aspartyl protease, which was purified from the H-gal-GP complex using gel filtration, with a reduction of 48 % in egg output and 36 % in worm burden. The protease was found to be located in the brush border of the worm's intestinal tract (Smith et al., 2003). Immunisation with a cocktail of proteases from *H. lineatum*, including a serine protease reduced the average number of the parasitic larval stage of the fly from 32 larvae in the control group of cattle to five larvae in the immunised group (Baron & Colwell, 1991). Recombinant cysteine proteases have also been used to immunise dogs against hookworm. The proteases used were located in the brush border of the hookworm intestine, as for *H. contortus*. The immunised group had a significantly lower egg count ($p \leq 0.05$) than the controls, although there was no significant difference in the number of worms recovered from the large and small intestine of vaccinated and control dogs (Loukas et al., 2004). The proteases that were located in the brush border either had their enzymatic activity inhibited (Newton & Munn, 1999; Smith et al., 1997) or were blocked by a physical antigen-antibody complex (Knox et al., 2003). The protein Bm91 was characterised in *R. microplus* and a recombinant form of the protein was used to immunise cattle, which were then challenged with ticks. The recombinant protein generated an immune response that reduced the number of feeding ticks by up to 52 % when compared to the control, non-immunised cattle (Riding et al., 1994). Further studies of Bm91 showed that it has a similar amino acid sequence to carboxydipeptidases (angiotensin converting enzymes [ACE]) of mammals and is active in the convertional assay for ACE, which uses hippuryl-Gly-Gly as a substrate. It can also be inhibited by the drug Captopril and by 5 mM EDTA (Jarmey et al., 1995). Proteinase inhibitors have also been used to block proteinase activity, causing an increase in mortality of *D. gallinae* fed blood

containing a range of inhibitors (McDevitt et al., 2006). Proteinases cleave proteins into smaller peptides or single amino acids for use by the organism, and blocking the protein's function causes disruption to the organism that can increase mortality. Proteolytic enzymes such as these are therefore suitable candidates for vaccines in *D. gallinae*, especially considering their role in bloodmeal digestion (Nisbet & Billingsley, 2000).

The SSH library also contained four contigs, identified from seven sequences that were homologous to ribosomal proteins. More protein synthesis would be required during moulting, reproduction and egg-laying, which the mites require a bloodmeal to perform.

Roche 454 sequencing technology allowed the formation of a large *D. gallinae* EST database in a very short space of time. This is especially useful when working with an organism that has not yet had its genome completely sequenced. The EST database formed from the Roche 454 sequencing described in this chapter contained 13,363 contigs. The genome of *Caenorhabditis elegans*, a free-living nematode, has been completely sequenced ($\sim 10^8$ basepairs [bp]) and annotated, and has approximately 19,000 predicted genes (Hashmi et al., 2001). The tick *Ixodes scapularis* has a genome size of approximately 2.15^9 bp (Ullmann et al., 2005) and has 24,785 genes described in the NBCI database (as of 27 June 2009). With the similarity observed in protein homology between tick species and *D. gallinae*, the number of genes expressed in the mite could be close to the total number of genes expressed in *I. scapularis*. With a redundancy in the database of 30 %, the 13,363 contigs in this EST database could represent approximately a third of the genes of *D. gallinae*.

The EST database generated by Roche 454 sequencing was too large to be analysed manually like the initial EST databases. The contigs were analysed using a semi-automated process using BLAST to compare the contigs to the NCBI nr protein database and to assign identities to each contig; 26 % of the contigs had homology to known proteins. The contigs were then BLAST analysed against the COG database

that allocated the contigs into groups based on inferred function (Figure 4.3). The group containing the highest number of contigs was Cellular Processes and Signalling (45 %). Of the top 100 contigs that had significant homology to a known or predicted protein, 63 % were classified as Post-translational modification, protein turnover or chaperone proteins. The top three most significant hits in this group were contigs homologous to heat shock proteins. Heat shock proteins are up-regulated when an organism is under stress. They are also involved in monitoring the proteins within a cell, including chaperoning proteins to the proteasome to be recycled and folding proteins into their three dimensional structure (Tutar & Tutar, 2010). High turnover of proteins would be expected after feeding since there is a different expression profile of genes. Some of these differentially expressed genes have been identified in this study by sequencing an SSH library (4.3.2) and by using Illumina Solexa sequencing (4.3.4) to count the number of each transcript in RNA samples extracted from fed and starved mite populations.

Other contigs in this group were cathepsin proteases. Cathepsin L, which has been used successfully in vaccination trials against parasites, was present in the database. It is a cysteine protease and has implied function in the moulting process of the filarial parasite *Onchocerca volvulus* (Lustigman et al., 2004) and feeding and immune evasion in *Fasciola hepatica* (Dalton et al., 2003). Multiple forms of the protein have been found that exhibit different tissue expression patterns and are expressed at different life stages (Irving et al., 2003). Native cathepsin L molecules have been purified from *F. hepatica*, the liver fluke, and were used in a vaccine trial that reduced parasite burden in the host by between 38.2 % and 69.5 % (Dalton et al., 1996). Vaccine trials have also been carried out using different recombinant versions of *F. hepatica* cathepsin L. Immunisation of rats with recombinant cathepsin L and B, another cysteine protease, either singly or in combination, significantly reduced the parasite burden by at least 50 % (Jayaraj et al., 2009). RNA interference has also been used to interrupt the function of cathepsin L in the filarial parasite *O. volvulus*. This interruption to the function of cathepsin L resulted in the failure by the parasite to shed its cuticle and complete the moulting process; 86 % of the parasites failed to moult compared with between 29 % and 62 % in the controls (Lustigman et al.,

2004). As described in 1.2.2, *D. gallinae* moults three times during its life cycle, two of which require a bloodmeal to occur. The use of cathepsin L as a vaccine may result in the inability of the mite to moult if the molecule identified is involved in this developmental process. The presence of cathepsin L activity in *D. gallinae* has previously been established, where reduction in specific enzymatic activity was observed when using specific inhibitors (Nisbet & Billingsley, 2000). The cysteine protease Der p 1 was found in the excreta of the house dust mite *Dermatophagoides pteronyssinus,* suggesting the protein has a digestive function (Chua et al., 1988) as an alternative to, or in addition to, a role in moulting. The protein is produced in greatest quantity in cells of the gut epithelia and stored in secretary vesicles. This localisation of the protein to the gut suggests that cathepsin L could potentially be a vaccine candidate (Dalton et al., 2003).

In the Roche 454 database there were also contigs that had significant homology to structural proteins such as tropomyosin, paramyosin, actin, tubulin and troponin T. Tropomyosin, actin and troponin T are all parts of the troponin complex, which is involved in muscle contraction (Ebashi et al., 1968). Recombinant troponin molecules have been used in immunisation trials against ticks, with mixed results. A troponin I-like protein of *Haemaphysalis longicornis* was characterised and a recombinant version was produced (You et al., 2001) and used in two immunisation trials. The first trial generated a protective immune response when rabbits were immunised with the recombinant protein. The ticks in the immunised group laid eggs that had a significantly lower weight than eggs laid by ticks in the control group. Also, adult and larval ticks took a significantly longer time to feed on animals immunised with the recombinant protein, but there was no significant difference for nymphs (You, 2004). During the second immunisation trial, mice were injected with the recombinant protein. Again a protective immune response was generated. The immune response caused a lowering of tick attachment rates from 98 % for the controls to 31.1 % for the test group (You, 2005). Troponin T has been cloned from the tick *H. qinghaiensis* and a recombinant protein was produced that was used with little effect in an immunisation trial. Sheep were immunised with the recombinant protein, generating a humoral response. However, the antibodies generated did not

cause an increase in tick mortality, and the mass of engorged adults and nymphs from the immunised group were not significantly different than the controls. The percentage of viable eggs laid was also higher in immunised groups (Gao et al., 2008). As described in chapter 5, paramyosin and tropomyosin have been successfully used to generate a protective immune response in hens against *D. gallinae* in immunisation trials.

The group containing contigs homologous to proteins involved in "metabolism" was the second biggest category (26 %). Vitellogenin is a protein found in the yolk of eggs of many species, which provides nutrition to the developing organism (Tellam et al., 2002). It was represented three times in the top 10 most significant hits in this group. Of this category, 183 contigs (21 %) were homologous to proteins involved in energy production and conversion. The mite is a very active organism and, as previously described, travels relatively large distances to find a bloodmeal, which requires a great deal of energy. Contigs that were homologous to kinases were also present in the metabolism group. Kinases add phosphate groups to proteins from donor molecules such as adenosine triphosphate (ATP) to substrate proteins that are part of metabolic pathways. Glycolysis is the process by which hexokinase phosphorylates glucose, using ATP as a phosphate donor molecule as it enters the cell, to glucose-6-phosphate (Caret et al., 1993).

Illumina Solexa sequencing was used in this study to count the numbers of each transcript in samples of RNA extracted from fed and starved mites, a process known as digital transcriptomics (Morrissy et al., 2009). The sequences were compared to the Roche 454 database to assign homology and a name to the count data. Only sequences with a count in all of the replicate samples were analysed to see if the gene expression was up- or down-regulated.

The majority of the 66 differentially expressed contigs (fold change > 3x) in this dataset were up-regulated in fed mites. However, there were four transcripts that were significantly down-regulated. Only one of the contigs that was down-regulated

in fed mites was homologous to a characterised protein, which was muscle myosin heavy chain.

The contig with the highest significant fold change was homologous to a vitellogenin gene (fold change of 110.853; $p < 0.001$); the second was also homologous to vitellogenin. Both these genes were up-regulated in fed mites. This result complemented that from the SSH library analyses, where vitellogenin was the most abundant gene. This is likely to have been due to a high percentage of adult female mites that were ovigerous following feeding.

Another contig that was present in the Roche 454 EST database and the SSH library, and was found to be up-regulated was homologous to cathepsin L. The up-regulation of a proteinase such as capthepsin L was expected as a large quantity of protein from the hen blood would be present in the mite cells, and the proteinase would be required to digest it (Delcroix et al., 2006). As previously stated, cathepsin L has a role in moulting (Lustigman et al., 2004), and coupled with its digestion function cathepsin L could be a potential vaccine candidate.

Experiments described in this chapter generated three EST databases that identified proteins that have been previously used in immunisation studies against various parasitic organisms, and that have been therefore selected by a rational process as potential vaccine candidates. The use of Roche 454 sequencing allowed the generation of a very large database. This database can now be further mined for previously described vaccine candidates from other parasitic species, as well as new candidates. A partial sequence of paramyosin was found in the Roche 454 database. Paramyosin has been used in vaccine trials against *Schistosoma japonicum*, where a recombinant form of the protein generated an immune response that reduced the adult worm burden of mice by up to 86 % (Ramirez et al., 1996). The full sequence for paramyosin was characterised and a recombinant version was used in an immunisation trial in chapters 5 and 6, respectively. The SSH library analysis found that vitellogenin was the most abundantly expressed transcript, and the transcripts counting function that can be employed after Illumina Solexa sequencing confirmed

that it was the most highly up-regulated transcript in fed mites. Using digital transcriptomics to count the exact numbers of each sequence in the samples gave a quantitative analysis which is not possible using an SSH library. The generation of the count data allowed statistical analysis to be performed, showing that there were significant differences in the levels of expression of genes between fed and starved mites.

As stated at the start of this chapter, there were five sequences identified in publically available *D. gallinae* databases in January 2008. There are now currently three EST databases with over 13,000 contigs derived from *D. gallinae*, of which over 25 % have significant homologies to proteins from other species. Using the new sequencing technologies such as Roche 454 and Illumina Solexa, the genetic sequence of *D. gallinae* could be determined and then annotated.

In summary, the experiments described in this chapter used four genetic sequencing approaches to identify possible vaccine candidates. Two EST databases were generated using material derived from total RNA; they were formed using classical EST techniques and Roche 454 sequencing technology to produce over 13,000 contigs. A second EST database was generated using an SSH library, to identify genes that were differentially expressed between fed and starved mite populations. The fourth dataset was generated using Illumina Solexa sequencing to quantitatively analyse the differentially expressed genes between fed and starved mites. The genes identified in the SSH library were confirmed as being differentially expressed in the dataset generated using Illumina Solexa sequencing. All four datasets identified proteins that could be possible vaccine candidates against *D. gallinae*.

Chapter 5:

Identification and characterisation of paramyosin and tropomyosin as potential vaccine candidates

5.1 Introduction

Recombinant proteins have been used successfully to immunise susceptible host organisms against many pathogens. Three protein antigens from the cell wall of *Coccidioides posadasii*, a soil-borne fungus that causes the human respiratory disease *Coccidioidomycosis*, were expressed and used to immunise mice. Of the vaccinated mice, which had been challenged with fungal spores, 90 % survived past 90 days whereas all the control mice died by day 20 (Tarcha et al., 2006). In the field of ectoparasitology, the vaccine TickGARD™ was developed with the recombinant protein Bm86 as the antigen (Rand et al., 1989). However, Bm86 has only been fully characterised in *Rhipicephalus microplus, R. annulus, R. decoloratus* and *Haemaphysalis longicornis* (Canales et al., 2008; Liao et al., 2007).

Two potential vaccine candidates for *D. gallinae* are paramyosin and tropomyosin. These proteins are powerful allergens (Nisbet & Huntley, 2006) that have been studied extensively worldwide as possible vaccine candidates against many parasite species, such as *schistosomiassis* (Chen et al., 2000; Ramirez et al., 1996), for paramyosin, and *Acanthocheilonema viteae* (Hartmann et al., 1997; Hartmann et al., 2006), for tropomyosin, both in their native form and as recombinant protein antigens.

5.1.1 Tropomyosin

Tropomyosin is a 41 kDa structural protein that is present in all types of eukaryotic cells. It is made up of two α-helical structures arranged as a coiled-coil and provides structural stability when joined end to end as part of the F-actin filament along with troponin, another actin filament binding protein. Tropomyosin has been found in many isoforms that have specific characteristics for the tissue in which they are found (Perry, 2001; Sereda et al., 2008). It is a flexible molecule that has regulatory properties in the sliding movement of actin filaments on myosin molecules. In the relaxed state, tropomyosin covers the actin filament inhibiting the binding of myosin.

When calcium ions bind to troponin the tropomyosin molecules moves allowing myosin to bind the actin filament.

The full sequence for tropomyosin has been identified in invertebrate species such as *Dermatophagoides pteronyssinus* (Asturias et al., 1998), *D. farinae* (Aki et al., 1995), *Psoroptes ovis* (Huntley et al., 2004) and *Dermanyssus gallinae* (Nisbet et al., 2006a). It is highly conserved, with the predicted *D. gallinae* protein having 89 % and 88 % identity to tropomyosin in two tick species, (*R. microplus* and *H. longicornis*) and 85 % identity to tropomyosin in the house dust mite (HDM) (Nisbet et al., 2006a).

Tropomyosin has been used successfully in immunisation trials to elicit a protective immune response. Tropomyosin from *A. viteae*, a filarial nematode, has been used to immunise jirds against a challenge infection. For the initial immunisation, the native form of the protein was used with Tween 80 and Pluronic, Squalan adjuvant, and reduced the worm burden by up to 64.4 % (Hartmann et al., 1997). Jirds were also immunised with a recombinant version of the protein, which elicited up to a 35 % reduction in adult worm burden (Hartmann et al., 2006).

However, immunisation of mice with DNA encoding the tropomyosin gene failed to provide protection against *Onchocerca volvulus*, even though a strong immune response was generated (Harrison & Bianco, 2000).

5.1.2 Paramyosin

Paramyosin forms the core of the thick filament of invertebrate muscles, only, unlike tropomyosin, it is not found in vertebrates (Elfvin et al., 1976). It is a larger protein than tropomyosin, with a molecular mass of approximately 100 kDa.

Paramyosin has been identified in many invertebrate species, including *R. microplus* (Ferreira et al., 2002), and has been identified as a possible vaccine candidate. The native form of paramyosin from S*chistosoma japonicum* was used to immunise mice,

which were then challenged with the parasite. The reduction of adult worm burden in immunised mice compared to control mice was between 62.3 % and 86.0 % (Ramirez et al., 1996). Recombinant fragments of *S. japonicum* paramyosin were expressed and used to immunise mice, which were challenged after the final immunisation. All recombinant fragments reduced worm burden by between 23.1 % and 39.9 %, and reduced the number of eggs present in the host liver by between 25.7 % and 77 .9 % (Zhang et al., 2006).

5.1.3 Aims of chapter

The aim of the work described in this chapter was to use the rational approach to vaccine development. This consisted of selecting proteins known to be vaccine candidates for other species of parasite (namely tropomyosin and paramyosin) and identifying, characterising and expressing the proteins to be used in an immunisation trial to generate antibodies. The protective nature of the antibodies generated was tested in the *in vitro* feeding assay.

5.2 Material and Methods

5.2.1 Polymerase chain reaction (PCR)

PCR was used to amplify the genes of interest. The protocol for a standard PCR has been described in 2.2.8.

The Advantage 2 polymerase mix (Clontech) was used to amplify DNA that was later used as a template to express a recombinant protein or DNA that was sent for sequencing. The Advantage 2 polymerase mix (Clontech) was used since it contains a proof-reading polymerase. For PCR using RACE ready cDNA, constructed as described in 5.2.5, the Advantage 2 kit was also used and a typical reaction is shown in Table 5.1.

Table 5.1. Typical reaction using RACE ready cDNA and Advantage 2 polymerase mix.

Ingredient	Volume (µl)
Template (either 3' or 5' RACE ready cDNA)	2.5
Universal primer mix (UPM)	5
Gene specific primer (10 pmol)	1
10x Advantage 2 PCR Buffer	5
dNTP mix (10mM)	1
50x Advantage 2 Polymerase mix	1
PCR grade water	34.5

The primers used in the cloning of tropomyosin and paramyosin are shown in tables 5.2 and 5.3, respectively. The primers to amplify tropomyosin from a pGEM®-Teasy template, supplied by Dr A. Nisbet (Nisbet et al., 2006a), added *Not*1 and *BamH*1 restriction sites. The reaction to amplify tropomyosin had an extension time of 1 min and the reaction to amplify paramyosin had an extension time of 3 min. All other conditions were as described in either 2.2.8, or in tables 5.2 and 5.3.

Table 5.2. Primers used to clone tropomyosin are listed below, together with their sequence, Tm, direction and extension time.

Name	Sequence	Tm (°C)	Direction
DGTroFor2pet2	5’ TTCGGATCCGATGGAGGCCATCAAGAATAAGATG 3’	54	Forward
DGTroRev2pet2	5’ AGTGCGGCCGCGTATCCGGTAAGCTCGGCGAA 3’	56	Reverse

Table 5.3. Primers used to clone paramyosin are listed below, together with their sequence, Tm, direction and extension time.

Name	Sequence	Tm (°C)	Direction
Para454_Rev_Begin	5’ CCGCTACACGTTTGCCACGAAGGGCAGA 3’	66	Reverse
Para454_For_End	5’ GTCACCACCTCTACGGTGCCTGGCGG 3’	67	Forward
Para_For_Gap	5’ CCAGGAAGCCATCGCCGAGTTCC 3’	62	Forward
Para_Rev_Gap	5’ GCAACGACGTATAGCGTCAGCAAGGG 3’	63	Reverse
Para_start_For	5’ ATGACTAGAAGCAGCAAGTACGTGTAC 3’	58	Forward
Para_454_for	5’ CCCTTGACCAACTTGGTGTGTCG 3’	59	Forward
Para_454End_rev	5’ CATCTGCTGAGTGGTGGTCTCC 3’	59	Reverse

5.2.2 Restriction digestion of PCR products and pET-22b(+) plasmid DNA for ligation

Approximately 350 ng of PCR product (20 µl) and 700 ng of pET-22b(+) plasmid (Novagen) were digested with 10 *u* of *Not*1 enzyme (1 µl) (Promega) and 1 µl of 10x buffer D. The volume was made up to 10 µl with molecular biology grade water (Sigma). The reaction was incubated for 1 hr in a 37°C waterbath. The QIAquick PCR purification kit (QIAGEN®) was used to purify the DNA from the excess enzyme and buffer, as described in 2.2.13.

Approximately 200 ng of PCR product (15 µl) and approximately 400 ng of pET-22b(+) plasmid (15 µl), which had been digested with *Not*1, were then digested with *BamH*1 (1 µl; 10 *u*), diluted with 16 µl of 2x buffer E. The reaction was incubated for 1 hr in a 37°C waterbath. The QIAquick PCR purification kit (QIAGEN®) was used to purify the DNA from the excess enzyme and buffer, as described in 2.2.13.

5.2.3 Dephosphorylation of plasmid by shrimp alkaline phosphotase

The pET-22b(+) plasmid (30 µl) that had been digested sequentially with *Not*1 and *BamH*1 was dephosphorylated using 2 *u* (2 µl) of shrimp alkaline phosphotase (SAP) (Promega) and 3.5 µl of 10x buffer. The digestion was incubated at 37°C for 1 hr. The dephosphorylated pET-22b(+) plasmid was stored at -80°C until required.

5.2.4 RACE ready cDNA

The RACE ready cDNA was constructed by, and used with permission of, Dr A. Nisbet. The cDNA was constructed using the SMART RACE ready cDNA amplification kit (Clontech).

5.2.5 Cloning and characterisation of the paramyosin gene

A partial sequence for paramyosin was found in the Roche 454 EST database, as described in chapter 4. This was used as a template to design primers to clone the rest of the sequence. The start of the known sequence was used to design the reverse primer Para454_Rev_Begin, and the end of the known sequence was used to design the forward primer Para454_For_End. The 5' RACE ready cDNA was used with the Para454_Rev_Begin primer to amplify the 5' end of the gene, and the 3' RACE ready cDNA was used with the Para454_For_End primer to amplify the 3' end of the gene. The UPM was used to extend the sequence in both directions. Figure 5.1 shows the position of the starting primers along with the UPM for the forward and reverse reactions.

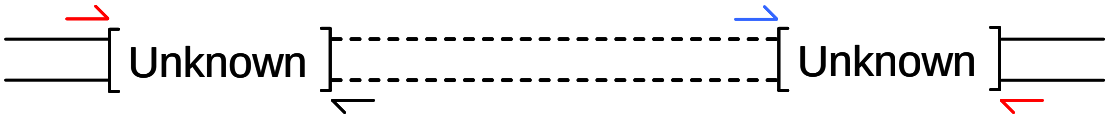


Figure 5.1. Diagram of initial primer sites for first PCR to extend the sequence of paramyosin. The dashed line represents the known part of the sequence from Roche 454 sequencing. The red primers are the UPM binding sites, the blue primer is Para454_For_End and the black primer is Para454_Rev_Begin. The unknown sequence would be filled in by PCR and Sanger sequencing.

A second PCR reaction was set up, as described in 2.2.8, to amplify the 5' end of the paramyosin gene using the gene-specific primer Para454_Rev_Begin and the UPM, as shown in figure 5.1. The 5' RACE ready cDNA was used as a template and the number of cycles was increased from 25 to 30. Negative control reactions were also set up by replacing the UPM or the gene-specific primer with PCR grade water (Sigma) as negative controls.

Four bands were cut out of the agarose gel and the DNA was extracted using the QIAquick gel extraction kit (QIAGEN®). All four DNA samples, 5', 3' A, 3' B and 3' C, had very low concentrations of approximately 2.1 ng/µl. A PCR reaction was set up as described in 2.2.8 with the number of cycles increased from 25 to 30. The primers used in reaction 1 were Para454_Rev_Begin and the UPM and the extracted

DNA sample 5' was used as template. The primers used in reactions 2, 3 and 4 were Para454_For_End and the UPM, and the extracted DNA samples 3' A, 3' B and 3' C, respectively were used as templates.

A ligation was set up using the pGEM®-T vector and T4 ligase, as described in 2.2.19. The construct plasmids were used to transform JM109 competent cells (Promega), as described in 2.2.10. After transformation, five white colonies were picked for samples 5', 3' B and 3' C and three white colonies were picked for sample 3' A. These colonies were used to inoculate LBroth (10 ml) with 10 µl ampicillin (25 µg/µl stock). The cultures were incubated at 37°C overnight with shaking. The plasmids were purified using the Wizard *Plus* SV miniprep DNA purification kit (Promega), as described in 2.2.11.

A gap of unknown sequences was left between the two contigs representing the 5' and 3' ends of the paramyosin gene. To sequence across this gap in the sequence, two primers were designed: Para_For_Gap and Para_Rev_Gap, as described in 5.2.1. These primers were used in two PCR reactions as described in 2.2.8, one with the 5' RACE ready cDNA using the gene-specific primers Para_For_Gap and Para_Rev_Gap and the other with the 3' RACE ready cDNA using the gene-specific primers Para_454_for and Para_454End_Rev.

Primers were designed at the start and the end of the gene, excluding the termination codon, to allow the addition of a His-tag to be added by the pET-22b(+) plasmid, to amplify the whole gene: Para_start_For and Para_454End_Rev, as described in 5.2.1. Two PCR reactions were set up using Advantage 2 polymerase mix (Clontech), with primers Para_start_for and Para_454End_rev to amplify the full length paramyosin open reading frame (ORF) from both the 5' and 3' RACE ready cDNA. This full length amplicon was ligated into the pGEM®T vector, as described in 2.2.19, and transformed into JM109 competent cells, as described in 2.2.10. Three colonies were picked and a PCR reaction was set up using primers Para_start_for and Para_454End_rev to check the transformation. The PCR amplicon from one colony

reaction was purified using the QIAquick PCR purification kit (QIAGEN®), as described in 2.2.13.

5.2.6 Expression of paramyosin and tropomyosin protein

Tropomyosin had previously been cloned into pGEM®-T (Nisbet et al., 2006a) and this was used as the template for expressing the protein in pET-22b(+). A PCR was performed that used the primers DGTroFor2pet2 and DGTroRev2pet2 through 30 cycles, and that used the pGEM®-T plasmid containing the tropomyosin amplicon as a template.

The digested amplicons for tropomyosin and paramyosin were ligated into pET-22b(+) and pET-SUMO plasmids respectively. The pET-22b(+) plasmid had been digested with both *Not*1 and *BamH*1 and then dephosphorylated. The ligation reactions were described in 2.2.19.

These constructed plasmids were first transformed into JM109 competent cells (Promega) to grow enough plasmid for the inefficient transformation into BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene), which were used to express the proteins. Transformations were carried out as described in 2.2.10. One colony from the first transformation into JM109 was grown in 10 ml of LBroth and 10 µl of ampicillin (25 µg/µl stock) overnight at 37°C with shaking. The plasmid was extracted using the Wizard SV plasmid purification kit (Promega). A PCR reaction was set up, as described in 2.2.8, to confirm transformation of the cells (data not shown). Another transformation was carried out using BL21-CodonPlus® (DE3)-RIL competent cells.

A single colony was grown in 10 ml of LBroth with 10 µl of both ampicillin (25 µg/µl stock) and chloramphenicol (50 µg/µl stocks) added overnight at 37°C with shaking. Glycerol stocks of BL21-CodonPlus® (DE3)-RIL cells transformed with plasmid containing either of the two genes were stored at -80°C, as described in 2.2.20. The overnight culture was used to inoculate two 200 ml preparations of

LBroth in 1 L baffled conical flasks. D-glucose (10 ml, 100 mg/ml stock) was added to stop expression of the protein before induction, and 200 µl of both ampicillin (25 µg/µl stock) and chloramphenicol (50 µg/µl stock) were added. The cultures were incubated for 3 hr at 37°C with shaking. The optical density (OD) was measured using the Cecil spectrophotometer at a wavelength of 600 nm. Once the OD reached 0.4, a 1 ml aliquot was taken as a pre-induction sample; the sample was centrifuged at 14,200 x *g* on a bench top centrifuge the supernatant was removed and the cell pellet was stored at -20°C. Isopropyl β-D-1-thiogalactopyranoside (IPTG) (400 µl of a 0.5 M stock) was added to each culture to induce protein expression. The cultures were incubated for a further 3 hr at 37°C with shaking. Another 1 ml aliquot was taken as a post-induction sample after the incubation, and treated the same as the pre-induction aliquot. The cultures were then split into 50 ml Falcon tubes and centrifuged at 3000 x *g* for 15 min at 4°C to pellet the cells. The supernatant was discarded and the pellet was stored at -80°C until required.

The proteins were extracted from the cell culture that expressed paramyosin pre- and post- induction samples. The cell pellets were thawed and re-suspended in 1 ml of binding buffer without urea, as described in 2.1.6. The cells were sequentially frozen at -80°C then thawed in a waterbath at 42°C three times. The cell suspension had 100 µl Triton X100 (20 %) in binding buffer without urea added and was mixed by inversion. The aliquots were centrifuged at 14,200 x *g* at RT. The supernatant contained the soluble proteins from the culture and the pellet contained all of the insoluble proteins. The supernatant (soluble fraction) was stored at -80°C until required. The insoluble protein pellet was re-suspended in 100 µl of binding buffer without urea. Once partly into solution, 900 µl of binding buffer with urea was added and as much of the pellet as possible was solubilised. The tubes were centrifuged at 14,200 *g* for 10 min. The pellet was discarded and the supernatant, which contained the urea soluble proteins, was stored at -80°C until required. A PAGE gel was run to visualise the proteins to determine how the protein was expressed, soluble or insoluble, as described in 2.2.3.

Following these initial analyses, larger scale extractions were performed as follows: A cell pellet from a 50 ml culture was re-suspended in 5 ml of binding buffer with no urea. Cells were sonicated on ice for two 20 sec bursts. The sample was incubated at -80°C for 10 min then thawed. Triton X100 (20 %) in binding buffer without urea (500 µl) was added and the sample was incubated on ice on a rocker for 30 min. The bacterial culture was transferred into 1.5 ml tubes and centrifuged at 14,200 x *g* for 10 min. The soluble proteins were in the supernatant, which was stored at -80°C until required, and the insoluble proteins were in the pellet. The pellets were re-suspended in 100 µl binding buffer without urea and then 900 µl of binding buffer with urea was added. The pellet was solubilised and centrifuged at 14,200 x *g* for 10 min. The supernatant, containing the urea-soluble protein, was decanted into a 1.5 ml tube and stored at -80°C. The remaining pellet was discarded.

A PAGE gel was prepared showing the protein profile of the soluble and urea-soluble fractions of the cell culture expressing the recombinant proteins.

5.2.7 Purification of His-tagged urea-soluble protein using His-trap columns (GE-Healthcare)

A 5 ml His-trap column (GE-Healthcare) was used to purify the His-tagged protein from the bacterial proteins. Five 1 ml urea-soluble protein fraction aliquots were purified at a time using the following protocol.

The pump tubing was washed with dH_2O and the flow rate was set to 5 ml/min. The column was attached to the pump using an adaptor. The column was washed with 15 ml dH_2O. Binding buffer with urea (15 ml) was used to equilibrate the column. The urea-soluble protein fraction (5 ml) was applied to the column using a syringe. An aliquot of 100 µl was retained as a pre-purified control (Sample A). The column was sealed top and bottom and placed on a rocker for 30 min at RT. The column was placed vertically on a stand to settle for 10 min. The column was washed with 25 ml of binding buffer without urea. The first 5 ml of elute was retained as Sample B, which consisted of protein that didn't bind to the column. Elution buffer 1 (5 ml),

described in 2.1.6, was applied and the 5 ml elute was collected (Sample C). Elution buffer 2 (5 ml) described in 2.1.6, was applied and the 5 ml elute from the column was collected (Sample D). Elution buffer 3 (5 ml), described in 2.1.6, was applied and the 5 ml elute from the column was collected (Sample E). Elution buffer 4 (15 ml), described in 2.1.6, was applied and the 15 ml from the column was collected in three 5 ml aliquots (Samples F, G and H). The column was washed with dH_2O, recapped and stored at 4°C. The samples were stored at -80°C until required.

The fractions were analysed by PAGE.

5.2.8 Dialysis of urea and imidazole from protein

Dialysis was used to remove the urea and imidazole buffer that was used to purify the recombinant protein. Purified protein (5ml) in 8 M urea and 500 mM imidazole was added to a section of dialysis tubing. A buffer with 2 M urea was prepared and the dialysis tubing was submerged and incubated overnight at 4°C. The protein in the 2 M urea buffer was removed and stored at -80°C until required.

5.2.9 Raising of antibodies against recombinant paramyosin and tropomyosin in Blackrock hens

An immunisation study was carried out to raise antibodies against the recombinant paramyosin and tropomyosin. Four Blackrock hens were sourced at 16 weeks of age and housed in two floor pens under standard environmental and feeding conditions for four weeks prior to the study commencing. The hens began producing eggs at 19-20 weeks of age. At 20 weeks of age, the hens were immunised with either recombinant paramyosin or tropomyosin. Each dose comprised 75 µg of recombinant protein (1 µg/µl), 200 µg of QuilA adjuvant (200 µg/µl), and PBS in a final volume of 300 µl. The injections were administered into alternate breast muscles of the hens. Following the primary injection, two booster injections were administered at two week intervals.

The experiment was carried out in accordance with the Animals (Scientific Procedures) Act 1986. The experiment was ratified by the ethics committee at Moredun Research Institute.

5.2.10 Immunolocalisation of tropomyosin and paramyosin in sections of *D. gallinae*

The antibodies raised in 5.2.9 could not be used to probe sections of *D. gallinae* since the mites in the sections had fed shortly before use so would have IgY in the gut. This would be detected by the anti-chicken IgY/HRP-labelled secondary antibody required to detect the antibodies raised. Sections of *D. gallinae* prepared for immunolocalisation, as described in 2.2.7, were probed with sera generated against *Blomia tropicalis* paramyosin (Blo t 11) and *Dermatophagoides pteronyssinus* tropomyosin (Der p 10). Table 5.4 shows the names and sources of the primary antibodies used.

Table 5.4. Antibodies used in the immunolocalisation, with references.

Antibody	Reference
Mouse monoclonal IgG anti-Blo t 11	(Ramos et al., 2003)
Mouse polyclonal IgG anti-Der p 10	(Huntley et al., 2004)

The anti-paramyosin antibody used in the immunolocalisation was raised against Blo t 11 and recognises paramyosin of other species of mites since, the protein is highly conserved.

For immunolocalisations, the mouse monoclonal IgG anti-Blo t 11 was diluted 1:200 and the mouse polyclonal IgG anti-Der p 10 was diluted 1:1000.

The secondary antibody used was rabbit anti-mouse IgG/HRP conjugate (Sigma) at a dilution of 1:2000.

5.3 Results

5.3.1 Cloning tropomyosin into expression plasmid pET-22b(+)

Figure 5.2 is a photograph of a gel showing the tropomyosin amplicons amplified by PCR using the *D. gallinae* tropomyosin in the pGEM®-T Easy vector (used with permission of Dr. A. Nisbet) as a template. The tropomyosin amplicon purified using the QIAquick PCR purification kit (QIAGEN®) described in 2.2.15, that was digested using the restriction enzymes *Not*1 and then *BamH*1, as described in 5.2.2, had a concentration that was determined to be 69.1 ng/µl by nanodrop.

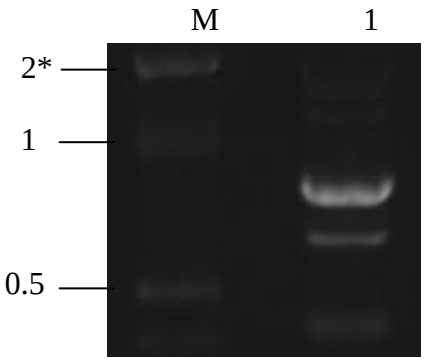


Figure 5.2. The tropomyosin amplicon after amplification by PCR from pGEM®-T Easy construct. Lane M: 1 kb DNA markers (Invitrogen). Lane 1: 5 µl of PCR product was run. Tropomyosin amplicon is the intense band smaller than 1 kb. The size was consistent for the tropomyosin gene. * kb.

5.3.2 Characterisation and cloning of paramyosin

Figure 5.3 is a photograph of a 1 % agarose gel that shows the PCR product sizes from the initial PCR reaction to clone paramyosin from 5' RACE ready cDNA, as described in 5.2.4. Three DNA molecules were amplified from the 3' RACE ready cDNA (lane 2) and were extracted using the QIAquick gel extraction kit (QIAGEN®), as described in 2.2.15. These bands were called 3' A, 3' B and 3' C.

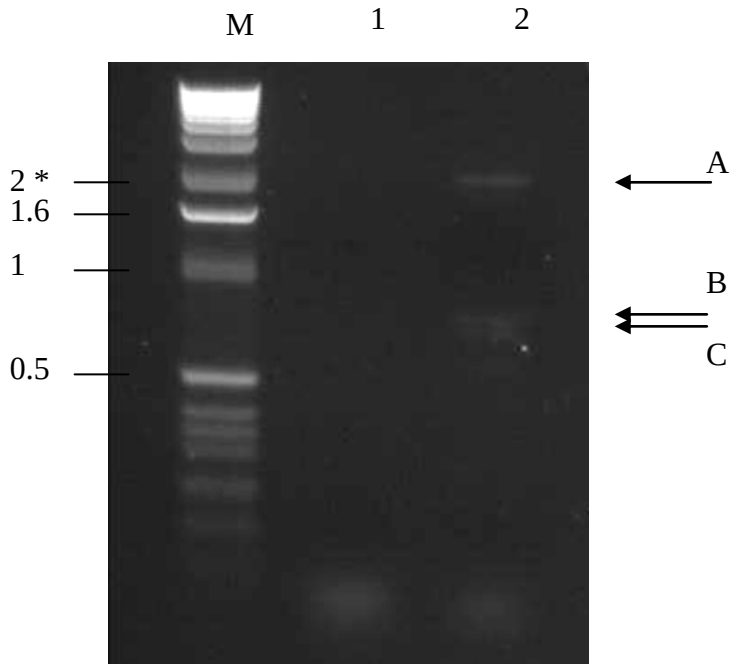


Figure 5.3. 5' and 3' RACE ready cDNA amplified by PCR using primers from both ends of the paramyosin gene sequence from the Roche 454 database to find the unknown intervening sequence. Lane M: 1 kb DNA markers (Invitrogen). Lane 1: 5 µl of product using 5' RACE ready cDNA as a template, no bands were present. Lane 2: 5 µl of product using 3' RACE ready cDNA as a template, bands marked A, B and C were cut out and purified. * kb.

Figure 5.4 is a photograph of a 1 % agarose gel showing the size of DNA molecules amplified during the second PCR reaction using the primers Para454_Rev_Begin and the UPM, and the 5' RACE ready cDNA as a template, as described in 5.2.5. In figure 5.4, the DNA amplified in the reaction with both primers can be seen in lane 1. Lane 2 contained only UPM and lane 3 contained only the gene-specific primer, Para454_Rev_Begin. As can be seen, there is a band present in the reaction with both primers (indicated with a white arrow in figure 5.4, which is absent from the single primer controls in, lanes 2 and 3. This band, named 5', was excised from the gel and then extracted using the QIAquick gel extraction kit (QIAGEN®), as described in 2.2.15.

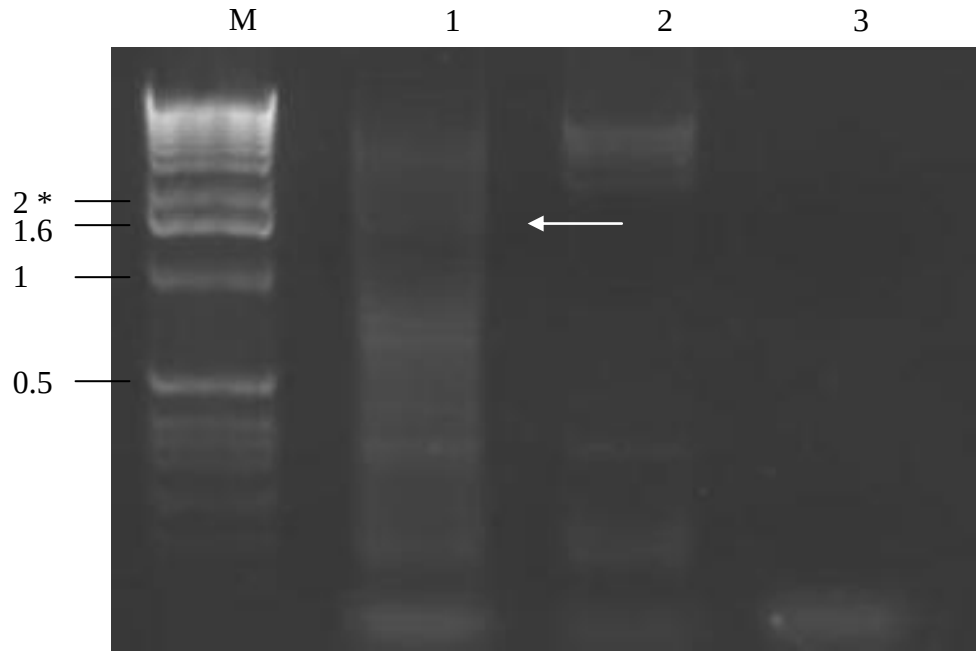


Figure 5.4. 5' RACE ready cDNA amplified by PCR using a gene-specific primer and the UPM to find the unknown sequence at the 5' end of the paramyosin gene. Lane M: 1 kb DNA markers (Invitrogen). Lane 1: Both UPM and gene-specific primer and 5' RACE ready cDNA as a template. Lane 2: As lane 1 but without the gene-specific primer. Lane 3: As lane 1 but without the UPM. The white arrow shows a band present in lane 1 that is absent in lanes 2 and 3. * kb.

Figure 5.5 is a photograph of a 1 % agarose gel showing the sizes of the DNA amplified by the PCR reaction to increase the quantity of DNA present in each sample, as described in 5.2.5. The bands marked with a white arrow in figure 5.5 were cut out and the DNA extracted using the QIAquick gel extraction kit (QIAGEN®), as described in 2.2.15. The concentration of the DNA extracted, which was measured by nanodrop, was higher than the first PCR, at approximately 30 ng/µl.

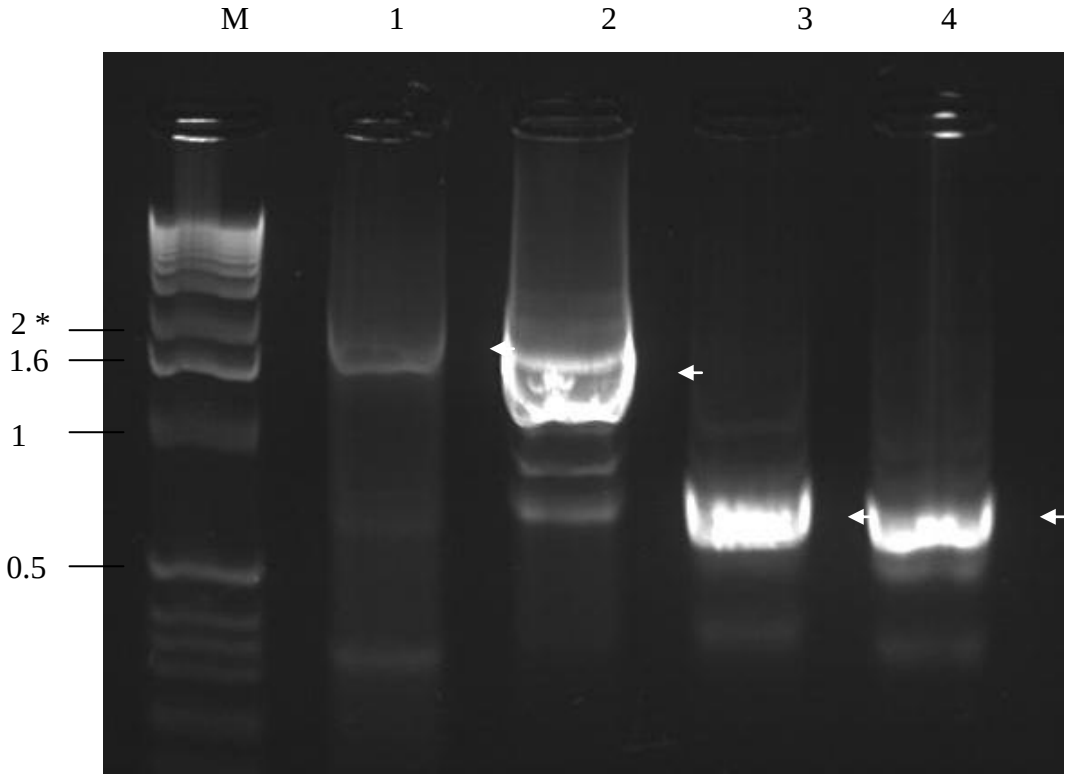


Figure 5.5. DNA, amplified by PCR to increase the quantity, was increased for ligation into the pGEM®T vector. Lane M: 1 kb DNA markers (Invitrogen). Lane 1: Sample 5'. Lane 2: Sample 3' A. Lane 3: Sample 3' B. Lane 4: Sample 3' C. The bands marked with a white arrow were cut out. * kb.

Figure 5.6 is a photograph of a 1 % agarose gel showing the size of DNA molecules amplified from the cloning sites of the constructed plasmids, as described in 5.2.5. As can be seen, the sizes of the DNA amplified were similar to that of the original product. There were inserts in all plasmids except those from colonies 3' A1 and 3' A2.

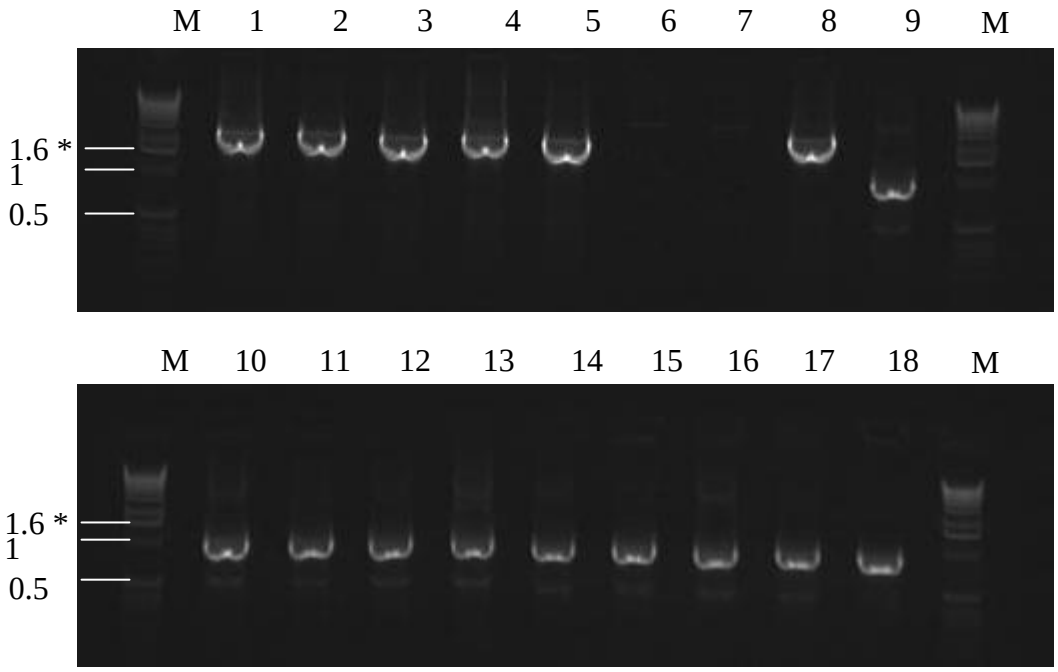


Figure 5.6. Insert DNA, amplified by PCR, from each of the colonies that had DNA from unknown sections of the paramyosin gene. Lane M: 1 kb DNA markers (Invitrogen). Lanes 1-18: Amplified DNA inserts. Lanes 1-5: DNA sample 5' colonies 1-5 respectively. Lanes 6-8: DNA sample 3'A colonies 1-3 respectively. Lanes 9-13: DNA sample 3'B colonies 1-5 respectively. Lanes 14-18: DNA sample 3'C colonies 1-5 respectively. As can be seen, there was no inserts in samples 3' A1 or 3' A2. The insert size was similar to that of the equivalent bands in figure 5.5. * kb.

Aliquots of plasmid extracted using the Wizard *Plus* SV Miniprep DNA purification system (Promega), as described 2.2.11, from the colonies that had a plasmid containing the gene were sent for sequencing at Eurofins MWG Operon, as described in 2.2.16.

The Lasergene software Seqbuilder (DNASTAR) was used to find vector sequence, gene-specific primer sequence or UPM sequence and to trim them off the nucleotide sequence obtained from MWG Biotech. A search of the NCBI non-redundant protein database was then performed on the remaining sequence using the blastx function. Samples 5' 1, 3, 4 and 5 represented paramyosin genes, as did the sequences from 3' B. The insert from samples 3' A, 3' C and 5' 2 were not paramyosin; 5' 2 was vitellogenin. The Lasergene SeqMan program (DNASTAR) was used to assemble the sequences from sample 3' B, along with sequences from plasmids 5' 1, 5' 3, 5' 4, 5' 5 and the sequence obtained from the Roche 454 sequencing, into two contigs;

contig 1 was 459 bases, which included the initiation codon, and contig 2 was 2100 bases that included the termination codon and the poly A tail.

Figure 5.7 is photograph of a 1 % agarose gel showing the size of the DNA molecules amplified by PCR using the gene-specific primers to amplify across the gap of missing sequence. The DNA molecule amplified in the reaction shown in lane 1 was the unidentified sequence between the two contigs. It was amplified using the gene-specific primers Para_For_Gap and Para_Rev_Gap from the 5' RACE ready cDNA. A DNA molecule with a size of approximately 1 kb was cut from the gel and extracted using the QIAquick gel extraction kit (QIAGEN®), as described in 2.2.15. The concentration was measured by nanodrop (4.7 ng/µl) and the DNA sample was sequenced by Eurofins MWG Operon. The DNA molecule amplified in the reaction shown in lane 2 was amplified from the 3' RACE ready cDNA using the gene-specific primers Para_454_for and Para_454End_Rev. A single band less than 1 kb was excised and extracted using the QIAquick gel extraction kit (QIAGEN®), as described in 2.2.15. The concentration was measured by nanodrop (7.1 ng/µl) and the DNA sample was sequenced by Eurofins MWG Operon.

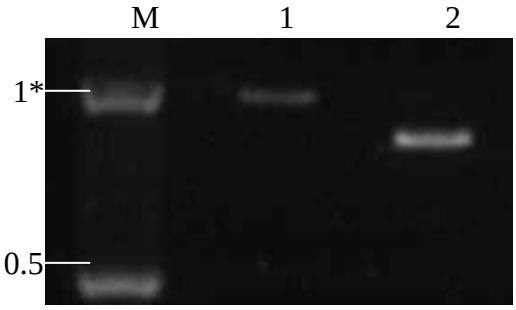


Figure 5.7. DNA amplified by PCR to sequence the unknown region between the two known contigs and the sequence generated by Roche 454 sequencing. Lane M: 1 kb DNA markers (Invitrogen). Lane 1: DNA amplified to sequence the gap between two contigs. Lane 2: DNA amplified to sequence the Roche 454-generated sequence. * kb.

All the sequences obtained by PCR described in 5.2.5 were trimmed and assembled using the Lasergene suite software into one contig that contained the sequence for paramyosin. The entire sequence was 3,329 bases long with an ORF of 2,622 bases. Figure 5.8 shows the full nucleotide sequence for the paramyosin gene, along with the positions of all the primers used in the characterisation and cloning of the protein.

```
1       ATGACTAGAAGCAGCAAGTACGTGTACAAGAGCTCTGGCGGCACCGCAGGTGACATCTCC
61      ATCGAGTATGGAACCGACCTCGGTGCCCTCACCAGGCTCGAGGACAAGCTCCGGCTGCTC
121     CAGGAAGATCTGGACAGTGAGCGGGAGATGAGGCAGAGGGTCGAACGTGAGAGGTCCGAT
181     CTACAATGCCAACTGATCGCACTCTCCGATCSTCTGGAGGAGGCCGAGGGAGGCGCCGAG
241     ACCCAGGTTGAGATGAACAAGAAGCGCGATACTGAGCTCTCAAAGCTCAGGAAGCTGCTC
301     GAAGATGTGCATCTGGAGAGCGAAGAGACTGCTCACCACCTGCGTAAGAAGCACCAGGAA
361     GCCATCGCCGAGTTCCAGGAGCAACTCGATGCYGTCGCCCGTGCTAAGGCAAAGTCTGAC
421     AAGGAGAAGCAAAAGTTCCAAGCTGAGGTGTACGAGCTGTTGGCCCAGGTGGAAAATACC
481     CAAAAAGAGAAGATGACGGCAATGAAGACTGTGGAGAAGCTCGAGACAACTGTTTATGAG
541     TTGAACATCCGCATCGAGGAGCTTAACCGAACGGTCACCGAGGTTACTGCCCAAAGGTCT
601     CGCCTCAGCGCTGAGAATGCCGAATATCTGAAGGAAATTCATGAGCTTAAGGTCTCCCTT
661     GATAACGTCAACCATCTGAAGGGCCAGCTGGCCACCCAGCTGGAGGATACACGCAGGAGG
721     CTCGAGGATGAGGAGAGGAGGCGCTCTACTCTTGAGAACCACATCCATACACTAGAAATC
781     GAGCTCGAGTCTCTAAAAGTACAACTGGACGAAGAGGCTGAGGCTCGCCTGGAAGTCGAG
841     AGACAGCTATCGAAGGCTAACGCTGATGCCGCTACGTACAAGAGCAAGTACGAAGCGGAG
901     TGCCAAGCCCATATCGATGAGGTCGAGGACCTTCGTCGTAAGATGGCTCAGAAGATCGCT
961     GAGTATGAAGAACAACTCGAAGCCCTTCTCAACAGGTGCTCGTCCCTGGAGAAGCAGAAG
1021    TCTCGTCTTCAGTCGGAGGTTGAGGTCTTAATTATGGACCTTGAGAAGGCAACAGCCCAT
1081    GCTCAGGCTCTCGAGAAACGCGTTGCCCAGCTTGAGAAGCTAAACAACGATCTCAAGGCC
1141    AAACTGGAGGAAGTTACTATGCTTATGGAGCAAGCCCAACGCGATGCTCGTCAAAAGGCC
1201    GCTGAGCTTGCCAAGCTGCAGCACGAATACGAAAAACTGCAGGAGCAGAAGGATGCTTTG
1261    GCCCGCGAAAACAAAAAACTCAGCGACGACCTTCAGGATGCCAAGAGCTCCCTTGCTGAC
1321    GCTATACGTCGTTGCCACGAGCTCGAAATCGAAATTAAGCGCCTTGAGAACGAGCGTGAT
1381    GAACTCAGCGCTGCTTACAAGGAGAGCGAGAGCCTAAGGAAGTCGGCTGAGAACCGCTGC
1441    CAGGTACTTCAATCCGAACTTCAGCAGGTTCGTCACGAGTACGAGAAGCGTCTGGCCCAG
1501    AAGGAAGAGGAGCTGGAGGCTCYGCGTAAGCAGTATCAGTTGGAGGTCGAAAGCCTAAAT
1561    ATGCGCCTTGCCGATGCCGAGGCGAAGCTCAAGACCGAAGTTGCCCGTATCAAGAAGAAG
1621    ATGCAGGCTCAGATCACGGAGCTCGAAATGTCTCTTGATGCTGCCAACAAGCAAAACCTT
1681    GRCCTGCAGCGGGTGATTAAGAAACAGGCCATGCAAATCCAGGAACTGCAGGCTCACTAT
1741    GACGAAGTGGCCCGACAGCTGCAGCAGACCCTTGACCAACTTGGTGTGTCGCAGCGAAGA
1801    TGCCAAGGCCTACAGGCTGAGCTCGATGAGCAGCGCGTAGCTCTTGAGTCTGCCCTTCGT
1861    GGCAAACGTGTAGCGGAACAGGCTCTCGAGGAGACGCAAGTTCGTGTTAATGAGCTTACC
1921    ACCATCAACGTCAACATCGCTTCAGCTAAGAGCAAGATCGAAGGCGAACTCTCTGCGCTT
1981    CAGGCAGACTACGACGAACTGCACAAGGAACTGCGCGTCGTTGACGAGCGCCTTCAGAGG
2041    ACACTTGTGGAGCTGAAGAGCACCAAGGATCTCTTGGTCGAAGAGCAGGAGAAGTACATC
2101    AAGTGCGAGTCCATTAAGAAATCTCTTGAAGTTGAAGTGCGTACGCTGCAGGTCCGCATC
2161    GAAGAGGTTGAAGCCAACGCACTGGCTGGCGGCAAGCGCGTCATTGGCAAGCTCGAGGCA
2221    AGGATTCGCGATATCGAGCTGGAGCTGGATGAGGAGAAGAAACACCACGGCGAAACTCAG
2281    AAGGTGCTTCGTAAGAAGGACCACCGCGCGAAGGAACTGCTTGTGCAGACTGAGGAAGAC
2341    CACAAGACCATCACAATGCTTAACGACACCATCGAGAAGCTCAACGAGAAGGTTAAGGTC
2401    TACAAGAGGCAGTTGAACGAGCAGGAAGGCCTCAGCCAGCAGAATCTGTCCCGAGTCCGT
2461    CGTTTCCAGCGCGAGCTGGAGGCTGCCGAAGACAGGGCCGACTCCGCTGAAGGAAATCTG
2521    TCTCTCATCAGGGCTAAGCACAGGAGCTGGGTCACCACCTCTACGGTGCCTGGCGGCAGT
2581    CGGCAGGTGTTCGTCTCTGAGGAGACCACCACTCAGCAGATGTAAGCGCTGCTGCATCTC
2641    CACCTACACACAATTCCAACAGTCTACAAGAGCACAGCCAGCAGCCATCGGGCCCGGGGT
2701    GACGCCTTTACTGAGCGTGGTACGACAATCACATAGCAACGTACGTCTAAGGACGTTTAC
2761    GATGTCTTCGCGACGCAACTTCTCTCAGCAAACGGCTGCACCCCATCAAATGCGGCAAAA
2821    GTGCGCCGTCCGGTTTCCTGGGCTAGCGGCGACCCTCCACAATCGAGTGCTCTCCACTTC
2881    CATAGACGCCCTCCCCCCCGCCGGCTGAACGCCTAGAGGCCGTGTCTCCGGGCGTCTTTA
2941    GCAGCAGAGTACATTCGATGTGCACGACGAGAGATAAAGCAGCGAACGAGCTCGACGGAG
3001    AGCGACAATGATACCAGCAACATCCCCATCTAAGGGGACCCGTCATCATGGGGTAGTTTG
3061    CTAGCCCTGCACTAAAGGGCCACTCAGCGTGGTTTGGCGATTTTTTTAGCGTCTAACTAT
3121    CTTTAACGCAAGATCTGTGCTAAGATCATTTACTGTTCTAAAACGGTTTTATTTGAGCCA
3181    AACTTTAAAAAAACGCATCAAAATTACGCCAGGTAGAAGCGCAAAAATCATCGCCTTTCC
3241    AGCGCCGCTTACAATAACGACAACGACAGAACAACAACGACAACAACAACAACCCCTA
3301    CCCAAAAAAAAAAAAAAAAAAAAAAAAA
```

Figure 5.8. The full nucleotide sequence for *D. gallinae* paramyosin. This figure shows the full nucleotide sequence for the paramyosin gene, from the initiation codon to the poly-A tail. Primer binding sites are highlighted in colour. The termination codon is in bold. Para_start_For is highlighted in purple. Para_For_Gap is highlighted in yellow. Para_Rev_Gap is highlighted in red. Para_454_for is highlighted in green. Para_454End_rev is highlighted in blue. Para454_For_End is highlighted in dark blue. Para454_Rev_Begin is highlighted in light green.

The complete paramyosin ORF was amplified by PCR, as described in 5.2.5. The amplified DNA had a size of ~3000 kb on a 1 % agarose gel (data not shown). The PCR amplicon from the reaction using the 5' RACE ready cDNA and a 3' RACE ready cDNA were excised and extracted using the QIAquick gel extraction kit (QIAGEN®), as described in 2.2.15. The DNA from each band was combined and the concentration measured by nanodrop (5.1 ng/µl).

The amplicons from the PCR reactions to test the transformation of JM109 competent cells with the paramyosin ORF inserts were the correct size in colonies 1 and 2, shown on a 1 % agarose gel (data not shown).

The plasmid from colony 2 was purified using the Wizard *Plus* SV miniprep (Promega), as described in 2.2.11. The concentration was measured by nanodrop to be 410 ng/µl. This plasmid was used in a transformation of BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene), as described in 2.2.10.

5.3.3 Expression and quantification of tropomyosin and paramyosin

Tropomyosin and paramyosin were expressed as described in 5.2.6.

Figure 5.9 is a scan of a PAGE gel prepared using an aliquot of total protein extracted by sonication from a BL21-CodonPlus® (DE3)-RIL cell pellet of a 50 ml culture, which had been induced to express tropomyosin. The bands around the 38 kDa molecular mass marker were cut out and given to the Proteomics Department at Moredun Research Institute for Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-ToF) analysis, described in 2.2.17, which confirmed that

tropomyosin was present in both samples. MALDI-ToF found peptides that covered 55.4 % of the amino acid sequence for tropomyosin in band A but only covered 27.8 % of the sequence in band B.

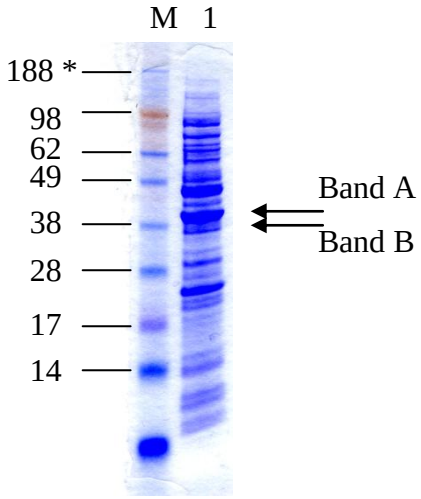


Figure 5.9. A PAGE gel showing the protein profile of the soluble proteins of BL21 cells after induction of the plasmid containing the tropomyosin gene. Lane M: SeeBlue plus 2 molecular mass markers (Invitrogen). Lane 1: The soluble protein profile from the BL21 cells after protein extraction. The gel was stained with SimplyBlue™ SafeStain. The two bands at a molecular mass of approximately 38 kDa. Band A and Band B were cut out and sent for MALDI-ToF analysis. * kDa.

Figure 5.10 is a scan of the PAGE gel showing the tropomyosin protein extracted in the soluble and insoluble phases. Tropomyosin was expressed at higher quantities in the insoluble phase, which was then extracted, as described in 5.2.7.

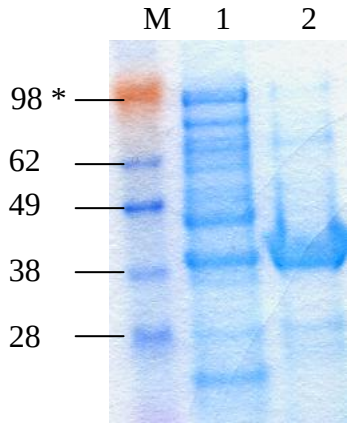


Figure 5.10. A PAGE gel showing the expression of tropomyosin in the soluble and insoluble phases. Lane M: SeeBlue plus 2 molecular mass markers (Invitrogen). Lane 1: The soluble protein profile from the BL21 cells after protein extraction. Lane 2: The insoluble protein profile from the BL21 cells after protein extraction. Tropomyosin can be seen as a band with a molecular mass slightly higher than 38 kDa. * kDa

Figure 5.11 is a scan of a PAGE gel showing the fractions from one of the purifications of tropomyosin. PAGE gels for the other purifications were similar (data not shown). Tropomyosin was eluted in protein fractions D and E in all the purifications.

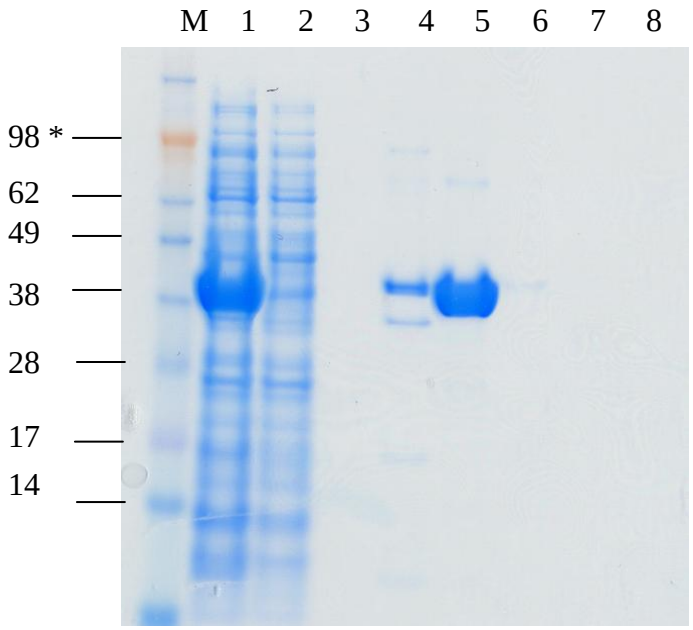


Figure 5.11. A PAGE gel showing protein fractions eluted during a purification of His-tagged tropomyosin expressed by BL21 competent cells. Lane M: SeeBlue plus 2 molecular mass markers (Invitrogen). Lane 1: Fraction A sample before binding to the column. Lane 2: Fraction B unbound proteins after first elution. Lanes 3-8: Fractions C-H with increasing concentration of imidazole. Tropomyosin released from the column over fractions D and E. * kDa.

Figure 5.12 is a scan of the PAGE gel that shows the pre- and post-induction samples taken from the BL21-CodonPlus® (DE3)-RIL competent cell culture expressing paramyosin. The bands at about 98 kDa in both the soluble and insoluble protein phases were cut out for MALDI-ToF analysis. Searches of the public databases Uniprot and NCBI nr databases were performed, as described in 2.2.17. After peptide mass fingerprint analysis, the *R. microplus* form of paramyosin was found to be the top hit in the insoluble protein fraction. Paramyosin was purified as described in 5.2.7 from the urea-soluble protein phase of the cell pellets.

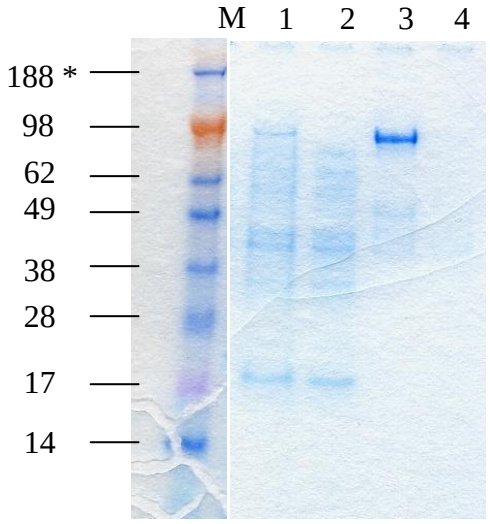


Figure 5.12. A PAGE gel showing the insoluble and soluble protein extracts of the pre- and post-induction samples of the paramyosin culture. Lane M: SeeBlue Plus 2 protein markers. Lane 1: Post-induction soluble proteins. Lane 2: Pre-induction soluble proteins. Lane 3: Post-induction insoluble proteins. Lane 4: Pre-induction insoluble proteins. Bands at 98 kDa in both lanes 1 and 3 were paramyosin; the bands were cut out for MALDI-ToF analysis to confirm. Paramyosin was expressed in greater quantities in the insoluble proteins post-induction.

Figure 5.13 is a scan of a PAGE gel showing the eight eluted fractions from one of the paramyosin protein purifications; the protein was eluted in the same fraction during the other purifications (data not shown). As can be seen, the protein was eluted from the column in fractions D and E with a very small quantity eluted in fraction F.

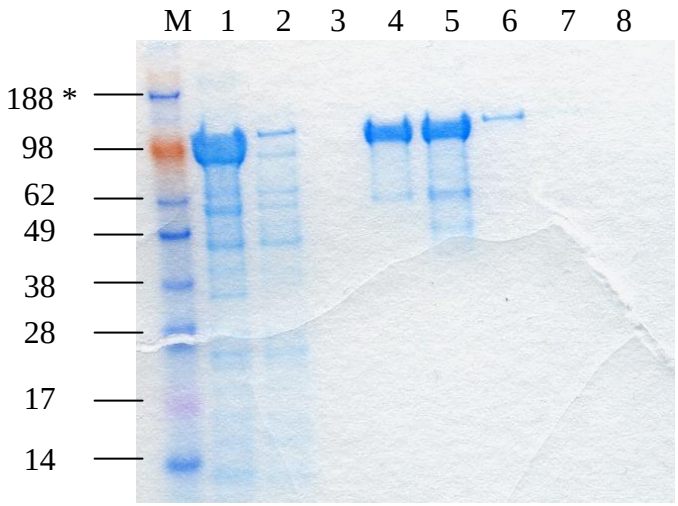


Figure 5.13. A PAGE gel showing protein fractions eluted during a purification of His-tagged paramyosin expressed by BL21 competent cells. Lane M: SeeBlue plus 2 molecular mass markers (Invitrogen). Lane 1: Fraction A sample before binding to the column. Lane 2: Fraction B unbound proteins after first elution. Lane 3-8: Fractions C-H with increasing concentration of imidazole. Paramyosin released from the column over fractions D and E. * kDa.

Fractions D and E that contained paramyosin were combined, and fractions D and E that contained tropomyosin were combined. The concentration of protein was measured in the two samples using the BCA assay (Pierce), as described in 2.2.1, to be 7 µg/µl for tropomyosin and 5 µg/µl for paramyosin. Both were diluted to a final concentration of 1 µg/µl.

5.3.4 Seroconversion of paramyosin and tropomyosin in Blackrock hens

Antibodies were raised against the two proteins using the protocol described in 5.2.9. Western blots, as described in 2.2.5, were prepared and probed with antibody raised against the recombinant forms of these proteins. Immunolocalisations, as described in 2.2.7, were prepared and probed with antibody raised in mice against Der p 10 and Blo t 11.

Figure 5.14 shows scans of Western blots of native *D. gallinae* proteins and recombinant forms of tropomyosin and paramyosin probed with antibodies raised

against recombinant *D. gallinae* tropomyosin and paramyosin. These proteins are not detected by antibodies from non-immunised hens. Bands can be seen at the correct molecular mass for tropomyosin in all lanes probed with the test sera. The recombinant form for paramyosin appeared to have break-down products. An intense band is detected at about 98 kDa, the correct molecular mass for paramyosin, along with numerous bands at smaller molecular masses down to approximately 40 kDa (lane 5 of panel B).

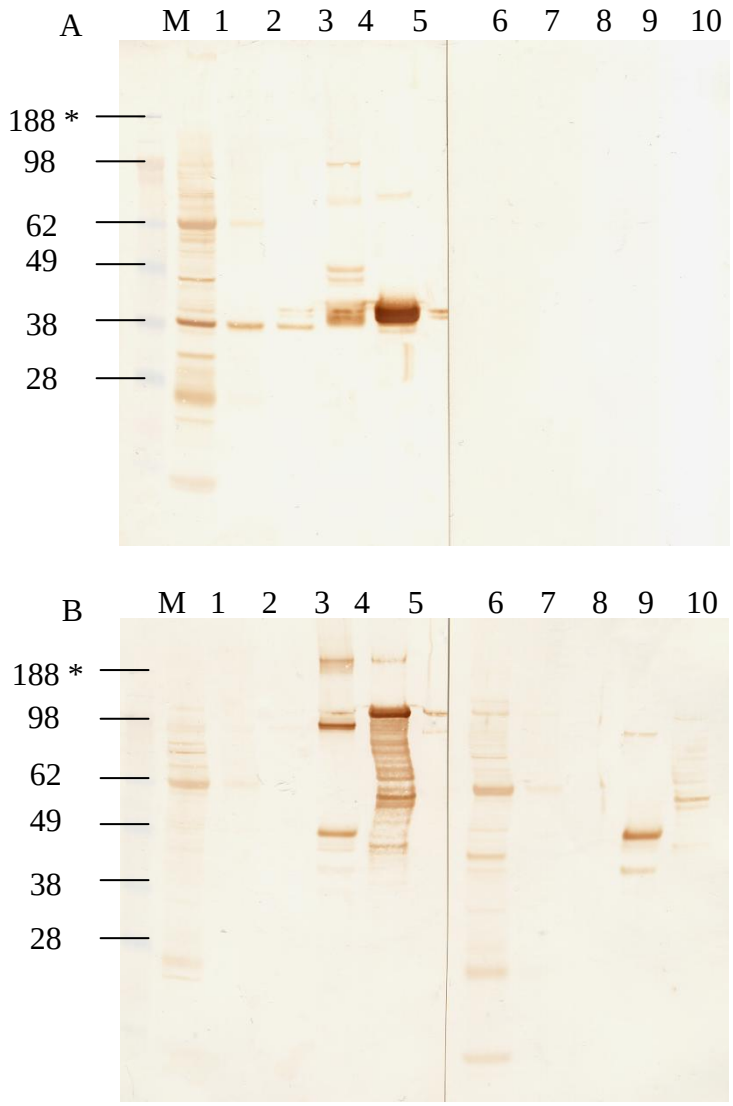


Figure 5.14. Scans of Western blots showing immunoreactive bands at the correct molecular masses for native and recombinant tropomyosin and paramyosin. Panel A: tropomyosin. Panel B: paramyosin. For both Western blots: Lanes 1 and 6: PBS-soluble proteins. Lanes 2 and 7: Membrane-associated proteins. Lanes 3 and 8: Integral membrane proteins. Lanes 4 and 9: Insoluble proteins. Lanes 5 and 10: Recombinant protein. Lanes 1-5 were probed with test sera and lanes 6-10 were probed with control sera. Lane M: SeeBlue plus 2 molecular mass markers (Invitrogen). * kDa.

Figure 5.15 shows three photographs of sections of *D. gallinae* probed with antibodies against Blo t 11 and Der p 10, as described in 5.2.10. A control section was probed only with the secondary antibody, a rabbit polyclonal anti-mouse IgG/HRP conjugate (DakoCytomation). There was a higher intensity of staining

present in the locomotory muscle bundles in the two test sections when compared to the control section.

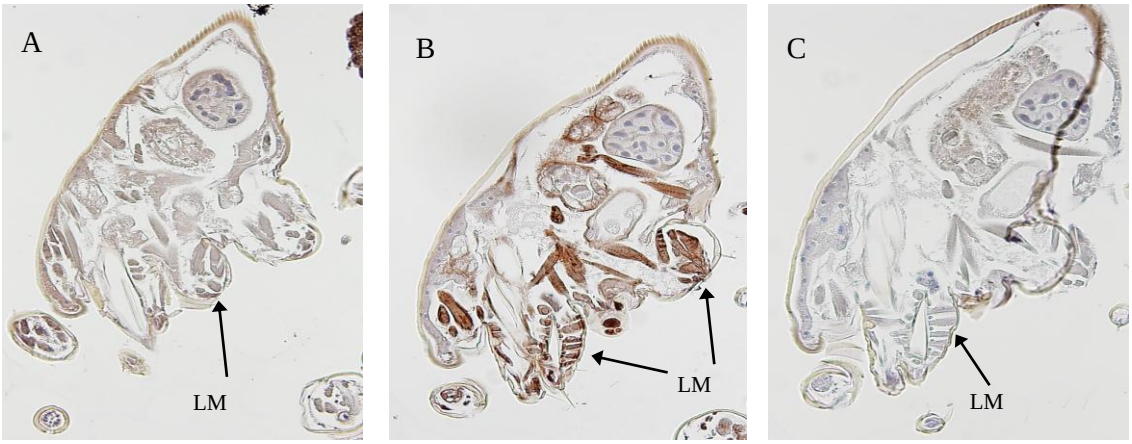


Figure 5.15. Immunolocalisations of sections of *D. gallinae* probed with sera raised against paramyosin and tropomyosin. Panel A: paramyosin. Panel B: tropomyosin. Panel C: No primary antibody control slide. LM = locomotory muscle bundles. Brown staining from DAB represents the presence of protein.

5.3.5 Assessment of paramyosin and tropomyosin as vaccine candidates by *in vitro* feeding assays

Three *in vitro* feeding assays were carried out on different dates to assess the protective capabilities of the antibodies raised against the two recombinant proteins along with a control group. The assay was set up as described in 2.2.6. Antibody was extracted from the yolk of eggs laid six weeks after initial immunisation for the test groups, as described in 2.2.4. Eggs from unvaccinated hens were collected and the IgY was extracted to act as controls. Fresh blood (2 ml) from Lohmann Brown hens, which had no prior exposure to mite antigens, was collected and mixed with 6 mg of antibody in solution (1 ml).

Figure 5.16 is a graph of the cumulative percentage mortality of the mites fed antibodies raised against tropomyosin and paramyosin against time in days. The mortality of the mites from the test groups was higher than that of the control groups. On day four the average percentage mortality was 33 %, 28 % and 9% in the

paramyosin, tropomyosin and control groups, respectively. The data was fitted to a simple logistic regression model, which individually compared the mortality from both test groups with that of the controls over the course of the experiment. This model gave a p value of ≤0.001 when the anti-tropomyosin antibody group was tested against the controls and a p value of 0.004 when the anti-paramyosin group was tested against the controls.

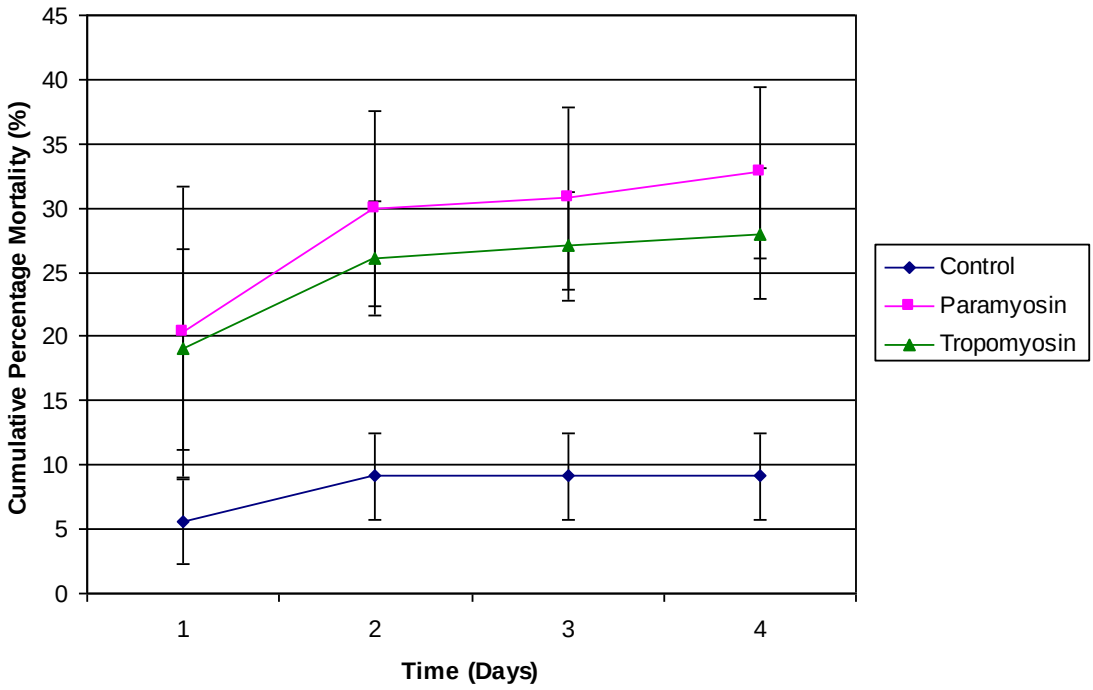


Figure 5.16. Cumulative percentage mortality of *D. gallinae* fed heparinised chicken blood enriched with antibodies raised against tropomyosin, paramyosin and negative control. Mortality was monitored daily over a four day period following feeding. Each point represents cumulative mean percentage mortality and is derived from data recorded in three independent experiments. A simple logistic regression model was fitted using three replicates per group. The *p* values for tropomyosin ≤0.001 and for paramyosin ≤0.004. The error bars represent the SEM.

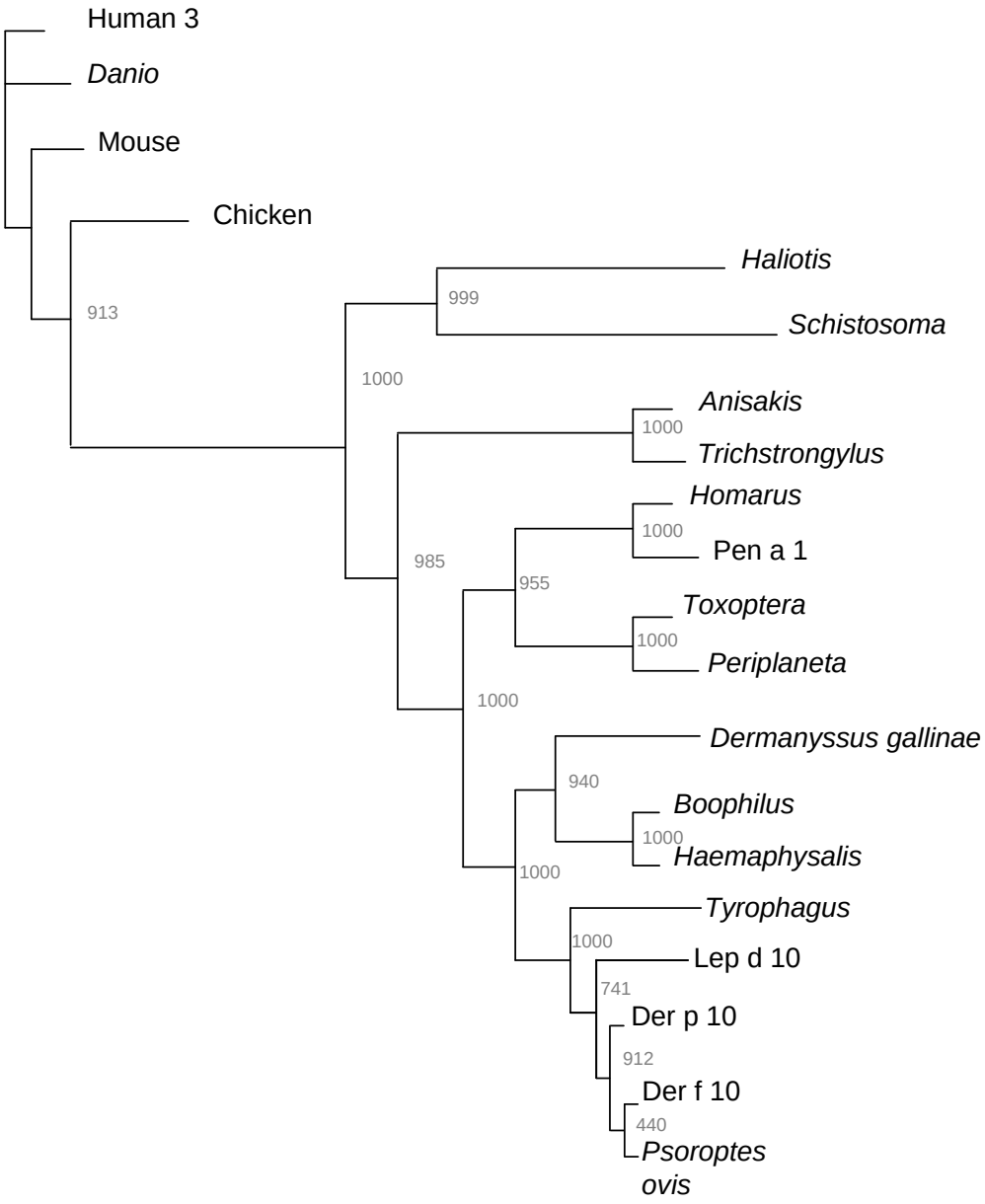
5.3.6 Phylogenetic analysis of paramyosin and tropomyosin

Phylogenetic trees were constructed showing the relative distance and genetic identity of the proteins compared to orthologues of the same protein in other species of mammal, fish, bird and other parasitic species. Clustal X was used to bootstrap the

neighbour-joining trees 1000 times based on the amino acid sequence of the protein, which were then viewed in TreeView (Page, 1996).

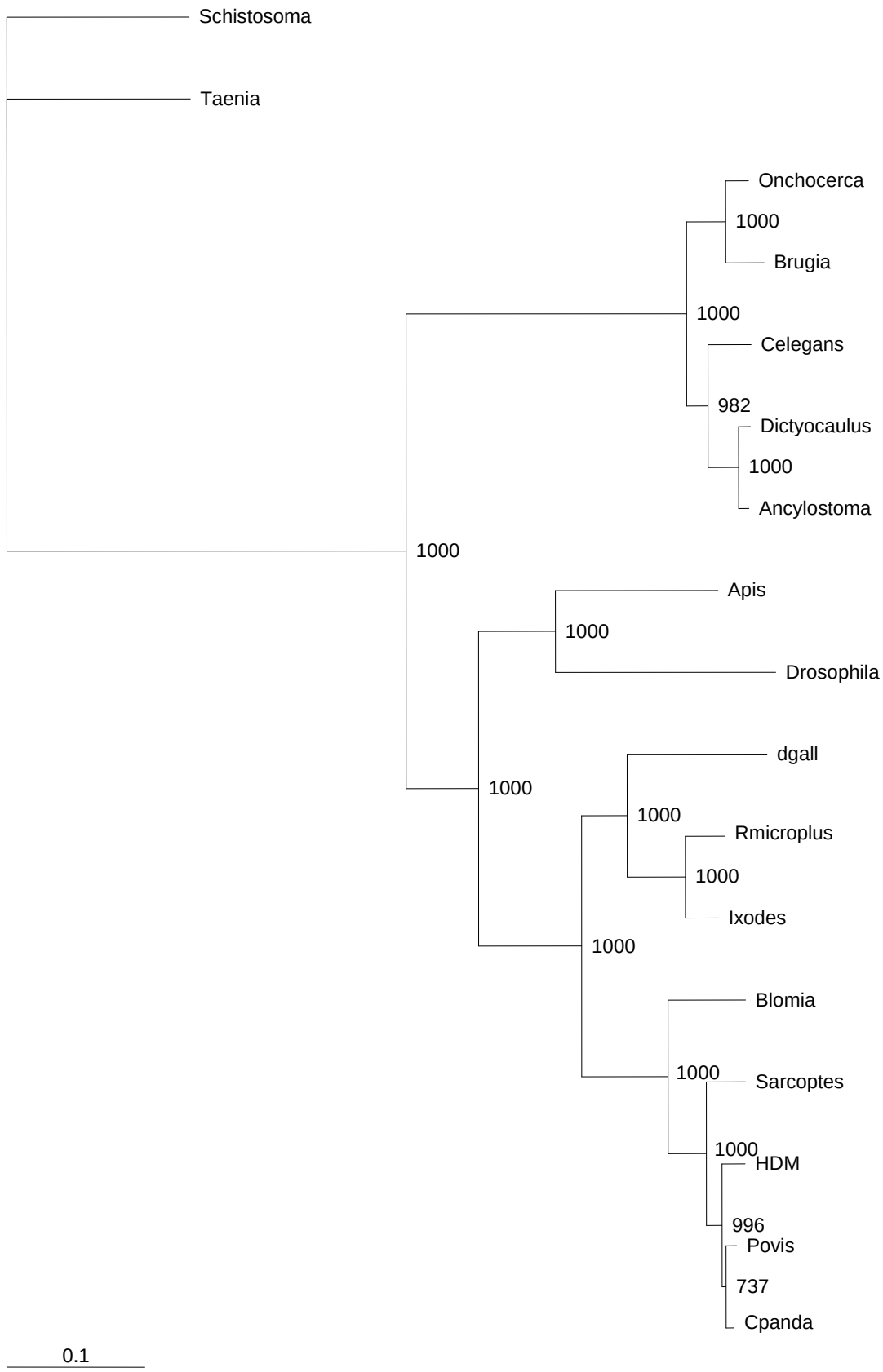
Figure 5.17 is a phylogenetic tree showing the relationship between the sequences of 20 tropomyosin molecules from different species. The tropomyosin sequence from *D. gallinae* was placed closely with those of two tick species, *R. microplus* and *H. longicornis*, with an amino acid sequence identity of 89 % and 88 %, respectively. The sequences for tropomyosin from four mite species were more distantly related. *D. farinae* and *Psoroptes ovis* both had an 85 % amino acid identity with *D. gallinae*.

Figure 5.18 is a phylogenetic tree showing the relationship between the sequences of 17 paramyosin molecules from different invertebrate species. The *D. gallinae* paramyosin sequence was again closely related to the tick species *R. microplus* and *Ixodes scapularis*, with which it had amino acid sequence identities of 83 % and 81 %, respectively. The identity of this sequence to the other mite species was 76 % for *Sacroptes scabiei* and 75 % for *P. ovis*.



Tropomyosin

Figure 5.17. Phylogenetic relationship between selected sequences for the tropomyosin protein from different species. The neighbour-joining tree was bootstrapped 1000 times using Clustal X and the resulting tree viewed with TreeView. Each node is annotated with a figure indicating the degree of bootstrap support for each branch. Abbreviations and Accession numbers are as follows: *Dermanyssus gallinae* (AM167555), chicken (*Gallus gallus* AAA48580), *Psoroptes ovis* (CAJ38272), Der p 10 (*Dermatophagoides pteronyssinus* AAB69424), Der f 10 (*Dermatophagoides farinae* Q23939), Lep d 10 (*Lepidoglyphus destructor* CAB71342), *Tyrophagus* (*Tyrophagus putrescentiae* AAT40866), *Haemaphysalis* (*Haemaphysalis longicornis* AAN05633), *Boophilus* (*Rhipicephilus* (*Boophilus*) *microplus* AAD17324), *Homarus* (American lobster *Homarus americanus* O44119), Pen a 1 (brown shrimp *Farfantepenaeus aztecus* AAZ76743), *Toxoptera* (brown citrus aphid *Toxoptera citricida* AAU84930), *Periplaneta* (American cockroach *Periplaneta americana* AAD19606), *Anisakis* (*Anisakissimplex* CAB93501), *Trichostrongylus* (*Trichostrongylus colubriformis* A44980), *Haliotis* (California red abalone *Haliotis rufescens* CAA53028), *Schistosoma* (*Schistosoma haematobium* Q26503), human3 (*Homo sapiens* tropomyosin 3 CAH71269), *Danio* (zebrafish *Dino rerio* NP_571180), Mouse (*Mus musculus* CAA46043). Reproduced with permission from Nisbet et al., 2006.


Schistosoma
Taenia
Onchocerca
1000
Brugia
1000
Celegans
982
Dictyocaulus
1000
Ancylostoma
1000
Apis
1000
Drosophila
dgall
1000
1000
Rmicroplus
1000
Ixodes
1000
Blomia
1000
Sarcoptes
1000
HDM
996
Povis
737
Cpanda
0.1

Paramyosin

Figure 5.18. Phylogenetic relationship between selected sequences for the paramyosin protein from different species. The neighbour-joining tree was bootstrapped 1000 times using Clustal X and the resulting tree viewed with TreeView. Each node is annotated with a figure indicating the degree of bootstrap support for each branch. Abbreviations and Accession numbers are as follows: dgall (*Dermanyssus gallinae*), Povis (*Psoroptes ovis* (CAJ38271)), HDM (*Dermatophagoides pteronyssinus* AAO73464), Rmicroplus (*Rhipicephalus* (*Boophilus*) *microplus* AAO20875), Schistosoma (*Schistosoma japonicum* AAD29285), Ancylostoma (dog hookworm *Ancylostoma caninum* ABC86903), Brugia (*Brugia malayi* Q01202), Sarcoptes (*Sarcoptes scabiei* AAK01181), Blomia (*Blomia tropicalis* AAM83103), Drosophila (*Drosophila melanogaster* CAA41557), Dictyocaulus (bovine lungworm *Dictyocaulus viviparus* AAT36324), Onchocerca (*Onchocerca volvulus* A48575), Apis (honey bee *Apis mellifera* XP_393281), Taenia (pork tapeworm *Taenia solium* AAK58494), Cpanda (*Chorioptes panda* ACB30406), Celegans (*Caenorhabditis elegans* CAA30857), Ixodes (black-legged tick *Ixodes scapularis* EEC01810).

5.4 Discussion

The experiments described in this chapter highlight the potential of two proteins, tropomyosin and paramyosin, to be used as a vaccine, either singly or as part of a cocktail of recombinant proteins along with other proteins such as histamine release factor, which has been shown to generate antibodies that significantly (p = 0.004) increase the mortality of mites (Bartley et al., 2009). To that end, the coding sequence of paramyosin has been identified (figure 5.8). Tropomyosin had already been characterised (Nisbet et al., 2006a), and both proteins were expressed as recombinant proteins (figures 5.11 and 5.13, respectively). The proteins were expressed in the insoluble fraction in the bacterial expression system. This could have been due to the large volume that the cultures were grown in. In future, cultures could be grown in a smaller volume to solubilise the proteins successfully. However, the proteins were diluted in PBS when the immunisations were formulated, so the concentration of urea injected into the hens was relatively small and did not seem to have a deleterious effect on the hens.

Antibodies generated against Der p 10 (tropomyosin) and Blo t 11 (paramyosin) were used to probe sections of *D. gallinae*, showing that homologous proteins recognised by the antibodies were present in locomotory muscle bundles, where the proteins would be expected to be expressed (figure 5.15). Antibody extracts from egg yolks collected from Blackrock hens immunised with the recombinant form of the two proteins were used in a Western blot and showed that a strong antibody response was generated (figure 5.14). This would be passed on to the chick as a large part of the early defence system (Rose, 1979). These antibodies were tested in the *in vitro* feeding assay described in chapter 3. There was a statistically significant increase in the mortality of the mites fed blood mixed with antibodies raised against the recombinant proteins, when compared to the control group (tropomyosin $p < 0.001$; paramyosin $p = 0.004$). This suggests that the antibodies generated bond to the proteins and interfere with their function to a significant degree. In the case of tropomyosin, these antibodies could inhibit the flexibility of the protein, which it requires to allow myosin molecules to bind actin. A fixed cross-linker was used to

form a stiff version of tropomyosin, which was found to inhibit the proper function in tropomyosin and allow actin filaments to slide on myosin molecules, even when calcium ions were bound to troponin (Mizuno et al., 2007). Paramyosin forms the core of the cytoskeleton filament so flexibility, to the same degree as tropomyosin, is not required. The antibodies that bind to paramyosin may disrupt the formation of the filament, which in turn would lead to a breakdown of the cellular cytoskeleton. The malformation of the cellular cytoskeleton could lead to cell death and the breakdown of the mite's gut, which could explain the characteristic "red leg" phenotype that can be seen in some of the dead mites observed (figure 3.10).

The cumulative percentage mortality of the mites that had been fed antibodies generated against the recombinant protein after five days for tropomyosin and paramyosin was 28.0 % and 32.8 % respectively. These figures are comparable to the 34.4 % reduction in *A. viteae* adult worm burden when tropomyosin was used to immunise jirds (Hartmann et al., 1997) and the reduction of *S. japonicum* burden of between 23.1 % and 39.9 % when paramyosin was used to immunise mice (Zhang et al., 2006). These are both endo-parasitic organisms and are continually under pressure from the host immune system. As discussed in chapter 3, this assay offers *D. gallinae* mites blood for only one night, which relates to one bloodmeal. During the course of the *D. gallinae* life cycle, a mite will feed multiple times, which exposes it to the host immune system. The percentage mortality observed could increase as mites feed multiple times.

There are many vaccines on the market that use recombinant proteins as the active component such as RECOMBIVAX HB®, which is a vaccine against Hepatitis B. The active antigen in the vaccine is a recombinant version of the surface antigen HBsAg. To date however, there have been only two recombinant protein vaccines on the market to control ectoparasites. These are TickGARD™ and Gavac™, which are used to control the tropical cattle tick *R. microplus*. TickGARD™ has now been withdrawn from the market in favour of using chemical control to the tick *R. microplus*. The active part of these vaccines is the gut-associated membrane-bound glycoprotein Bm86. It has now been characterised in other species of tick such

as *H. longicornis* (Liao et al., 2007) and *Boophilus annulatus* (Canales et al., 2008). Bm86 has not yet been characterised in *D. gallinae*. Probing a Western blot with the urea-soluble proteins extracted from *D. gallinae* with an antibody raised in rabbits against Bm86 shows an immunoreactive protein with a molecular mass of about 98 kDa (data not shown). The immunoreactive band was very specific with only a single band appearing on the lane of the Western blot. However, when the proteins around the region of the immunoreactive bands were excised and analysed by MALDI-ToF spectrophotometry, none showed any homology to Bm86. The quantity of *D. gallinae* protein loaded on to the gel may have been too low to have the Bm86 homology in *D. gallinae* present in sufficient quantity for the MADLI-TOF analysis to detect. MALDI-ToF analysis would only detect the proteins present in greater quantities. To obtain 3.3 mg of antigenic material, which would have included Bm86, 1.368 kg of ticks were processed (Willadsen et al., 1988). Therefore this protein only makes up a tiny fraction of the proteins present in the tick and possibly in *D. gallinae*. Although Bm86 has not yet been characterised in *D. gallinae*, there is evidence to suggest that there is a homologue present, and it could be identified by using the same protocol (Willadsen et al., 1988). However, the amino acid identity of a possible *D. gallinae* homologue of Bm86 could be as low as 37 %, which was the case for Ba86 (Canales et al., 2008). The low percentage identity could explain why degenerate primers designed from the sequences already present did not amplify the gene from *D. gallinae* cDNA (data not shown).

Although Bm86 has not been identified in *D. gallinae*, recombinant Bm86 and subolesin, a regulatory protein in control of multiple cellular pathways (Galindo et al., 2009), have been used to immunise hens. An *in vitro* feeding assay was then used to monitor and assess any protective qualities of the antibodies raised. The antibodies raised against these recombinant proteins significantly increased the mortality of the mites when compared to mites fed antibodies from a control group, but not to a degree where they could be used on their own as a vaccine (Harrington et al., 2009a).

The reduction in survival elicited by the antibodies generated against all the proteins discussed here have not been as clear cut as that of the effect of Bm86 used to

immunise cattle against *R. microplus*. Homologues of Bm86 and subolesin might not be present in *D. gallinae*. Since the mortality of the mites fed anti-tropomyosin and paramyosin antibodies was only a maximum of 28.0 % and 32.8 % respectively, these and other antigens may have to be combined to produce a cocktail vaccine that would offer a higher level of mortality in the mites.

Phylogenetic analysis of tropomyosin (figure 5.17) and paramyosin (figure 5.18) identified the species with the closest sequence identity to ticks rather than other mites. Amino acid homology with *D. gallinae* tropomyosin was highest in *H. longicornis* and *I. scapularis* for tropomyosin and paramyosin respectively. A protein homologous to the tick histamine release factor (HRF), which is expressed in many tissues of the tick, including the salivary glands, mid-gut, ovary and haemocytes, was identified in *D. gallinae* and expressed as a recombinant protein. A phylogenetic analysis of HRF showed that the *D. gallinae* protein was closely related to that in orthologous proteins from tick species of the order Ixodida (Bartley et al., 2009). The similarities in the genetic makeup of *D. gallinae* and ticks could be very important. If there is a close relationship between *D. gallinae* and tick it could mean that the vaccine candidates targeting tick species could be successful in *D. gallinae*. For example, Bm86 the antigen in TickGARD™, which has been reference throughout this thesis, was used to immunise hens. There was no significant difference between the mortality of mites in the test and control groups when *D. gallinae* were fed antibodies against Bm86 (Harrington et al., 2009a). However Bm86 has not been identified in many tick species and where it has been identified the sequence homology was very low. A Bm86 like protein in *D. gallinae* may perform the same role as in *R. microplus* but have a very different genetic sequence. This may be the case for other proteins identified in ticks as vaccine candidates.

The experiments described in this chapter have shown that tropomyosin and paramyosin are present in *D. gallinae* and an immune response, specifically an antibody response, is generated against them. When these antibodies were tested in an *in vitro* feeding system, they had a significantly deleterious effect on the longevity of *D. gallinae*.

Chapter 6:

Testing the protection afforded to hens after immunisation with a cocktail of recombinant proteins and a complex mix of PBS-soluble proteins from *D. gallinae*

6.1 Introduction

The experiments described in chapters 3 and 5 demonstrated that both the PBS-soluble fraction of proteins extracted from *Dermanyssus gallinae* (Wright et al., 2009) and two recombinant proteins, tropomyosin and paramyosin, generated antibodies that had a killing effect on *D. gallinae* when fed to mites in an *in vitro* feeding assay. The experiments described in this chapter used those proteins along with three additional recombinant *D. gallinae* proteins, cathepsin D, cathepsin L and histamine release factor (HRF) (Bartley et al., 2009), in an *in vivo* immunisation trial.

When working with an ectoparasitic species which spends all its time on a mammalian host, a vaccine trial is relatively simple to design. The trials which tested the vaccine TickGARD™ against the tick *Rhipicephalus microplus*, in cattle, were straightforward in design. The tick attaches to its host and can be easily monitored throughout the experiment. After the parasite has fed, it will be engorged with blood and will be easy to locate in the animal enclosure (Hope et al., 2010). The ectoparasite can also be restricted to specific area of the host animal. For example, rabbits were infested with the brown ear tick *R. appendiculatus*, the ticks could be restricted to the ears by the use of ear bags (Yamada et al., 2009). When working with *D. gallinae*, the main problem is containment. The mites can move freely between the cages in a battery system (personal unpublished observation) and are therefore difficult to retain for observation. Although the hen is a relatively small animal, experiments have been performed where specifically built containers with mites inside were attached to the back of hens (Arkle et al., 2009). The feeding rate of the mites fed using this *in vivo* method was compared to the feeding rate of mites using an *in vitro* feeding assay. The feeding rates of those fed using the *in vitro* feeding assay were higher than those fed using the *in vivo* system. However as, discussed in chapter 3, the reporting of some results in that study was inconsistent.

During the immunisation trial described in this chapter, the hens were injected with proteins known to generate a protective antibody response (PBS-soluble fraction), and a recombinant protein cocktail comprising of tropomyosin, paramyosin,

cathepsin D, cathepsin L and HRF, and then challenged with *D. gallinae*. To contain the mites, the legs of the cages were placed in buckets of water and diatomaceous earth, which can be used as a non-chemical control agent (Maurer et al., 2009), was piled around the blocks of cages to contain the mites within their designated treatment groups.

6.1.2 PBS-soluble fraction extracted from *D. gallinae*

The PBS-soluble fraction was included in this experiment as the proteins generated an anti-mite antibody response, which was demonstrated by use of the *in vitro* feeding assay experiments described in chapter 3. The PBS-soluble protein fraction was made up of a complex mix of proteins and other macromolecules. The antibodies raised against the PBS-soluble fraction, as described in chapter 3, recognised proteins with molecular masses from 17 kDa to 188 kDa. The PBS-soluble protein fraction has been shown to contain proteins such as tropomyosin and paramyosin, as described in chapter 5, which were also represented in the recombinant protein cocktail.

6.1.3 Recombinant protein cocktail

A recombinant protein cocktail was used to immunise one of the groups of hens in the trial. The recombinant proteins included tropomyosin, paramyosin, cathepsin D, cathepsin L and HRF. These proteins were expressed using either the pET SUMO expression system (Invitrogen) or the pET-22b(+) plasmids expressed in BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene).

Tropomyosin and paramyosin, which have molecular masses of 38 kDa and 98 kDa respectively, have been shown to generate antibodies that significantly increased the mortality of a population of mites that were fed blood containing the antibody in the *in vitro* feeding assay, as described in chapter 5. The mortality of the mites was increased from 9 % in the control group to 28 % in the tropomyosin group and 33 % in the paramyosin group five days after feeding.

Cathepsin D and L proteinases, which have molecular masses of approximately 45 kDa and 40 kDa respectively, were produced in recombinant form and used to generate antibodies by Dr. K. Bartley (Moredun Research Institute). The antibodies were fed to mites in the *in vitro* feeding assay and an increase in mortality was seen when compared to the controls. The mortality increased from 9 % in the controls to 32 % in the cathepsin D group and 18 % in the cathepsin L group (Bartley et al., unpublished). Proteinase inhibitors, which inhibit a broad range of enzymes such as serine, cysteine, aspartic and metalloproteinases, were added to hen blood fed to *D. gallinae* in the *in vitro* feeding assay. The results showed an increase in mortality of mites fed the proteinase inhibitors when compared to the control group (McDevitt et al., 2006).

HRF, which has a molecular mass of 23 kDa, has been identified in *D. gallinae* and the sequence has been obtained. Phylogenetic analysis of the sequence showed that it clustered with the homologous proteins in ticks, however this was the first description of the protein in a mite species. Antibodies were raised against HRF in hens and fed to mites in the *in vitro* feeding assay. There was a significant difference in the mortality (p = 0.004) between the control (2.8 %) and test groups (9.2 %) suggesting that the antibodies had a moderate killing effect on the mites (Bartley et al., 2009).

6.1.4 Aims

The purpose of the experiments described in this chapter was to test the protective capability of native mite PBS-soluble proteins and a recombinant protein cocktail (containing tropomyosin, paramyosin, cathepsin D, cathepsin L and HRF) *in vivo.* The protective nature of the antibodies generated in the hens was measured by monitoring the population of a *D. gallinae* challenge. Western blots were prepared with the proteins, probed with the antibody raised in the hens, and developed using DAB. A densitometric measurement of the intensity of the DAB staining was used as a measure of antibody titre.

Behavioural observations, egg production and hen plumage and skin condition were also measured during the study.

6.2 Methods

6.2.1 *In vivo* trial schedule

Seven weeks old Lohmann Brown hens (180 birds) were housed in two pens in a single room and wing tagged on arrival at SAC Auchincruive. The hens were allowed to acclimatise for four weeks.

At 10 weeks of age all the hens were then weighed, and allocated into three groups balanced for weight. Each group was then allocated to one of the three treatments, which were control group (adjuvant only), recombinant protein cocktail group (tropomyosin, paramyosin, cathepsin L, cathepsin D and HRF) or the PBS-soluble fraction group. The hens were injected with the proteins, prepared as described in 6.2.2, at 11 weeks of age. Booster injections were administered in weeks 14 and 17, as shown in figure 6.1. All the injections were administered into the hens' breast muscle, alternating sides for each injection. On week 16, the hens were places in cages. Each cage housed two hens, with 10 cages per block. The treatment groups were spaced evenly throughout the room and diatomaceous earth was formed into barriers around each block of cages and the legs of the cages were also placed in water buckets to stop the transfer of mites from one treatment group to the next, as shown in figure 6.2.

Blood samples were taken from the hens' brachial veins at 10, 17, 19, 23, 27 and 32 weeks of age for antibody titre monitoring and haematology measurements described below. The blood samples for antibody titre monitoring were centrifuged at 3000 x *g* for 15 min and the serum was removed and stored at -20°C until required.

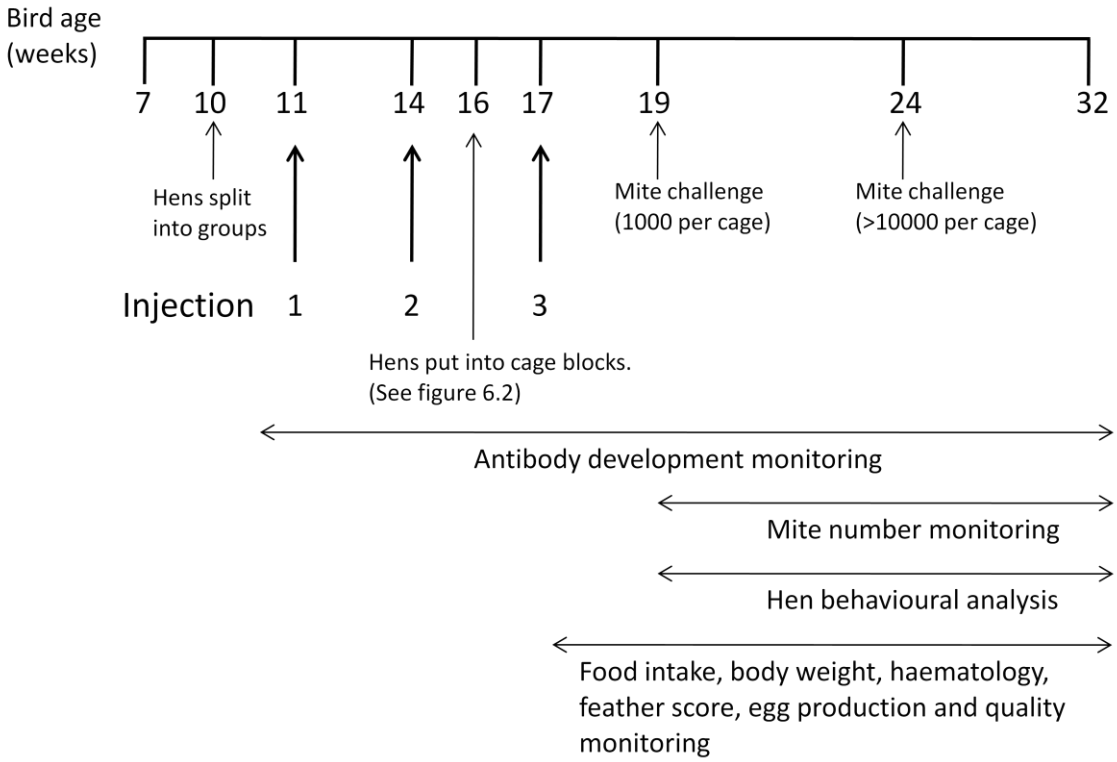


Figure 6.1. Chart of the vaccination and experiment timings of the *in vivo* trial.

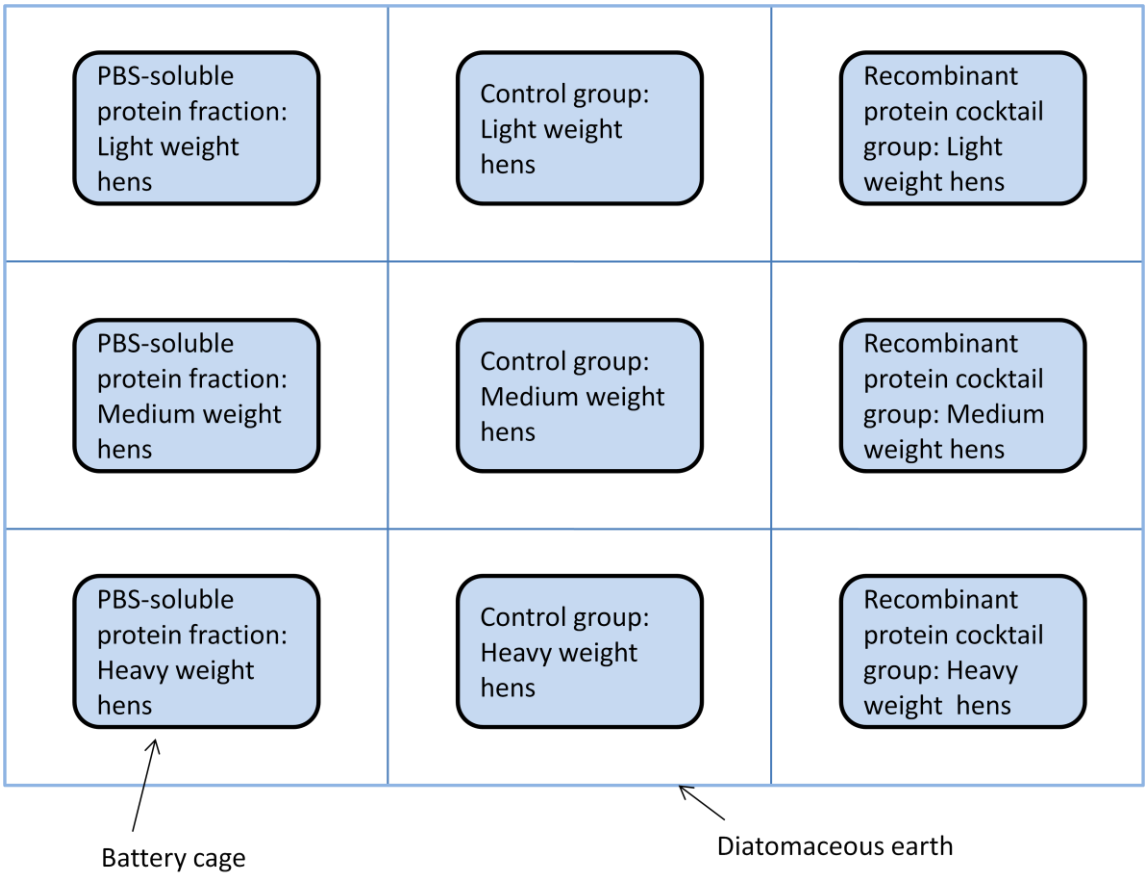


Figure 6.2. A diagram of the battery cage layout during the mite challenge portion of the *in vivo* experiment. Each group contained 10 cages, with two hens per cage.

The hens were challenged with 1000 mites per cage in week 19, prepared as described in 6.2.3, and in week 22, were challenged with more than 10,000 mites and eggs, per cage. The size of mite population was monitored in weeks 19, 21, 23, 25, 27, 29 and 31 using ADAS MiteMonitor traps, which were placed in the base of the cages. The mites collected were stored at -20°C until they were counted under a light microscope. Mite numbers were analysed using a generalised linear mixed model (GLMM), fitted using residual maximum likelihood (REML) to the mite counts to determine differences between the treatment groups.

The experiment was carried out in accordance with the Animals (Scientific Procedures) Act 1986. The experiment was ratified by the ethics committee at the Scottish Agricultural College in Auchincruive.

6.2.2 Immunisations dose preparation

The PBS-soluble proteins injected into the hens were prepared as described in the first part of 3.2.2. Each dose comprised of 75 µg of PBS-soluble mite extract (75 µl), 200 µg of QuilA (1 µl) and was made up to 500 µl with PBS (224 µl).

The recombinant proteins used in the trial were tropomyosin and paramyosin, which were expressed and purified as described in chapter 5; cathepsin D, cathepsin L, and HRF (Bartley et al., 2009). Each dose comprised of 25 µg of tropomyosin, paramyosin, HRF and cathepsin D (25 µl of each protein) and 10 µg of cathepsin L (10 µl), 200 µg of QuilA (1 µl) and was made up to 500 µl with PBS (389 µl).

The dose for the adjuvant only control group was made up with 499 µl of PBS and 200 µg QuilA (1 µl).

PAGE gels were prepared with the PBS-soluble protein (13 µg) or the recombinant protein cocktail made up of tropomyosin (2 µg), paramyosin (2 µg), cathepsin D (2 µg), HRF (2 µg) and cathepsin L (0.75 µg) as described in 2.2.3.

The immunisation doses were stored at -80°C until required. The doses were thawed and warmed to RT shortly before use.

QuilA, which is a saponin extracted from plants (Rajput et al., 2007), was used as the adjuvant since it has been used at Moredun Research Institute in the past, when administered at the correct dose has no side effects, it can enhance antibody production and can be used in low doses (Oda et al., 2000).

6.2.3 Challenge with *D. gallinae*

For the first challenge, *D. gallinae* collected fresh from a commercial poultry farm were split into 90 aliquots of roughly 1000 mites (0.13 g), based on weight, in universal tubes (50 ml) with vented caps (Corning). The mites were stored at 4°C

overnight. An opened tube and its cap were placed separately in the corner of each cage of hens to allow the mites to infest the cage blocks.

Due to the failure of the first mite challenge, the cages were re-challenged with a greater number of mites. The mites of the second challenge were added to the experimental cages with the battery cage debris they were collected in. Approximately 2 g of debris and mites of all life stages including eggs were placed in a universal tube (50 ml) with vented caps (Corning). The mites were added to the cages in the same manner as described in the first challenge. The number of mites added to each cage was estimated to be greater than 10,000.

6.2.4 Western blots and densitometry estimation of antibodies raised

Western blots were prepared as described in 2.2.5. Serum (10 µl), collected as described in 6.2.1 from each hen within a cage block (20 hens), was pooled for the primary antibody. A Western blot was prepared for each pooled sample from the immunised groups using either the PBS-soluble protein (10 µg of protein) fraction or the recombinant protein cocktail (tropomyosin (3 µg), paramyosin (3 µg), cathepsin D (3 µg), cathepsin L (1 µg) and HRF (3 µg)). Each Western blot was probed with both the test and control sera, which were diluted 1:100.

One lane on each of the Western blots was prepared with recombinant tropomyosin, which was probed with sera raised during the experiments, as described in chapter 5, as a positive control.

The antibody response was estimated by densitometry. The Western blots were scanned onto a computer and stored as JPEG files, with the dimensions 980 x 1118 pixels. The AlphaEaseFC program (Alpha Innotech) was used to measure the density of the DAB precipitated on the Western blot by the HRP-labelled secondary antibody.

The JPEG file containing the image of the Western blot was opened in this programme. The Analysis tool was used for one lane at a time, with a width setting of 17 and the Invert button set to ‘on’. Once the software had measured the average density down the lane, all the peaks automatically detected were removed, a horizontal base line was added and the whole length of the lane was selected to measure the area under the graph. The density was measured for each lane, including the tropomyosin positive control. To normalise the data, the densities of each lane were divided by the density of the tropomyosin positive control lane.

A two-sampled unequal variance t-test with two-tailed distribution was performed on the density measurements obtained from the Western blots at each time point, comparing the normalised PBS-soluble protein group data to the normalised control group data. The data from the recombinant protein cocktail group was analysed in the same manner.

ELISA could have been used to calculate the antibody titre however it would not have given an informative analysis of the molecular mass range of protein detected by the antibodies raised and due to project time constraints the testing of an ELISA protocol was not possible.

6.3 Results

6.3.1 PAGE gel of proteins used in immunisations

PAGE gels were prepared using aliquots of the PBS-soluble protein fraction and aliquots of the recombinant protein that was used in the recombinant protein cocktail. Figure 6.3 is a scan of the two PAGE gels on which the PBS-soluble proteins and the recombinant proteins were run. As can be seen, the PBS-soluble protein fraction was a complex mix of proteins with a wide range of molecular weights. The recombinant proteins used in the protein cocktail had been expressed and purified using a His-trap column as described in 5.2.4 and 5.2.5 respectively.

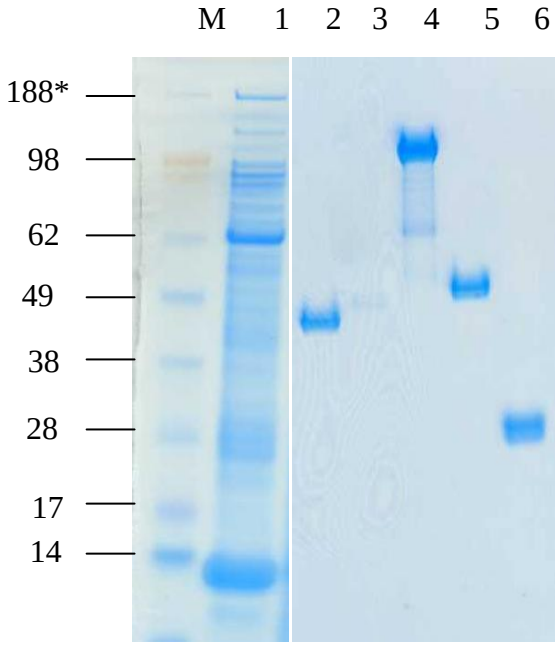


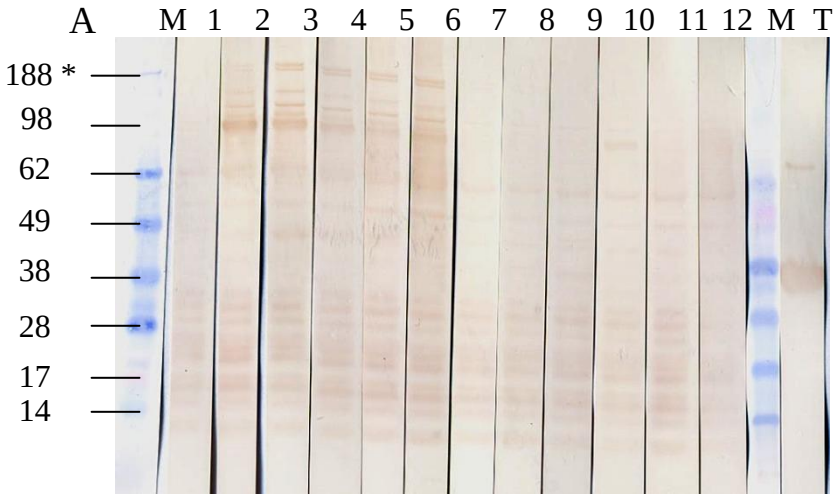
Figure 6.3. A scan of two PAGE gels on which PBS-soluble proteins and the recombinant proteins were run. Lane M: SeeBlue plus 2 protein markers. Lane 1: PBS-soluble protein fraction. Lane 2: tropomyosin. Lane 3: cathepsin L. Lane 4: paramyosin. Lane 5: cathepsin D. Lane 6: HRF.

Due to possible autoproteolysis activity by cathepsin L, the band was very faint on the gel. This could have lowered the concentration of intact protein in the immunisations described in 6.2.2. However immunisation with cathepsin L in previous studies (performed by Dr. K. Bartley) showed that even a low concentration of the protein was enough to generate an antibody response that when fed to mites in the *in vitro* assay increased the mortality of mites compared to controls.

6.3.2 Seroconversion and densitometry estimation of antibodies raised against injected proteins

6.3.2.1 PBS protein fraction group

Three Western blots were prepared with the PBS-soluble protein fraction from *D. gallinae* and were probed with the antibodies raised against the protein in the light, medium and heavy weight hen groups as described in 6.2.4. Figure 6.4 is a scan of the three Western blots. Panel A was probed with sera collected from block H (the heavy hens), panel B was probed with sera taken from block M (the medium weight hens) and panel C was probed with sera taken from block L (the light hens). As can be seen from all three Western blots, an antibody response was generated after the initial week 10 pre-bleed when compared to the controls. However, only the hens injected with PBS-soluble proteins in block L appeared to generate a stronger antibody response than the controls from block L.



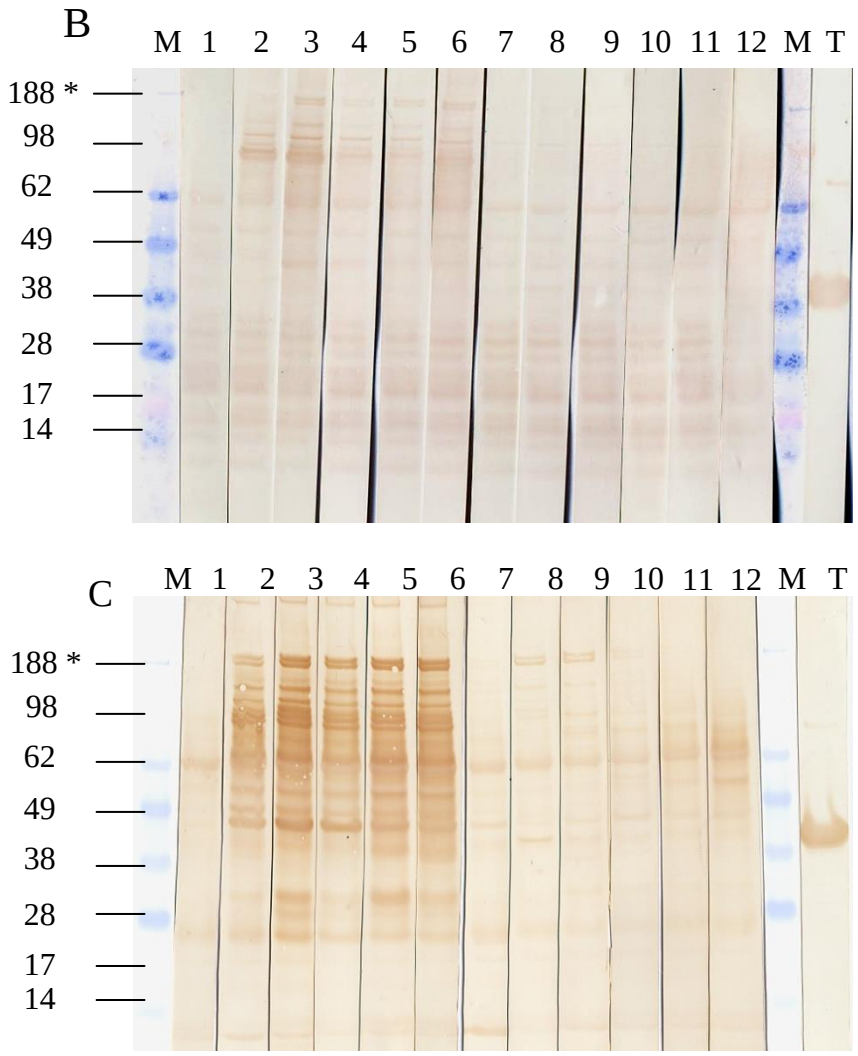


Figure 6.4. Western blots probing PBS-soluble protein with antibodies raised against PBS-soluble proteins and control antibodies for six time-points throughout the experiment. Lane M: SeeBlue plus 2 molecular weight markers (Invitrogen). Lane T: tropomyosin protein probed with anti-tropomyosin, as described in 6.2.5. The protein probed on lanes 1 through 12 for all three blots was ~13 µg of *D. gallinae* PBS-soluble protein. Panel A was probed with antibody from block H. Panel B was probed with antibody from block M. Panel C was probed with antibody from block L. Lanes 1 and 7: probed with antibody from week 10. Lanes 2 and 8: probed with antibody from week 16. Lanes 3 and 9: probed with antibody from week 18. Lanes 4 and 10: probed with antibody from week 22. Lanes 5 and 11: probed with antibody from week 26. Lanes 6 and 12: probed with antibody from week 30. * kDa

The average of the three normalised densitometry readings from each time-point of the Western blots (figure 6.4) was calculated. Figure 6.5 is a graph of the normalised densitometry measurements against time. A t-test was performed, as described in

6.2.4, which revealed that there was not a statistically significant difference between the control and test groups at any time point.

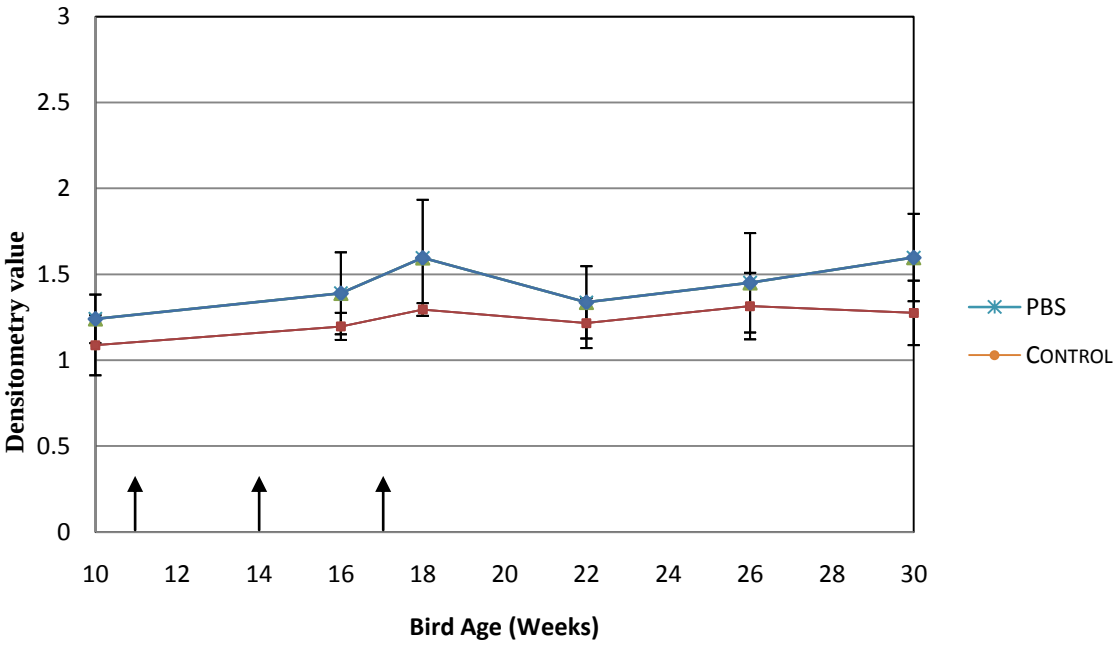


Figure 6.5. Graph showing the normalised intensities from the Western blots prepared with the PBS-soluble protein fraction. The densitometry values started between 0.75 and 1.5. Throughout the experiment there was no significant difference between antibody levels between all of the groups. The hens from the test group in the light hen block did generate antibodies that appeared to follow the same pattern as the antibody response against the recombinant proteins. Arrows indicate dates of immunisation. The error bars represent the standard error of the mean, n = 3.

6.3.2.2 Recombinant proteins group

Three Western blots were prepared with each of the five recombinant proteins used in the recombinant protein cocktail: paramyosin, tropomyosin, cathepsin D, cathepsin L and HRF. The blots were probed with the antibodies raised against the proteins in the light, medium and heavy weight hen groups during the immunisation experiment. Figure 6.6 is a scan of three Western blots. Panel A was probed with sera collected from block H, panel B was probed with sera taken from block M and panel C was probed with sera taken from block L. As can be seen from all three

Western blots, a strong antibody response was generated after the initial week 10 pre-bleed when compared to the controls.

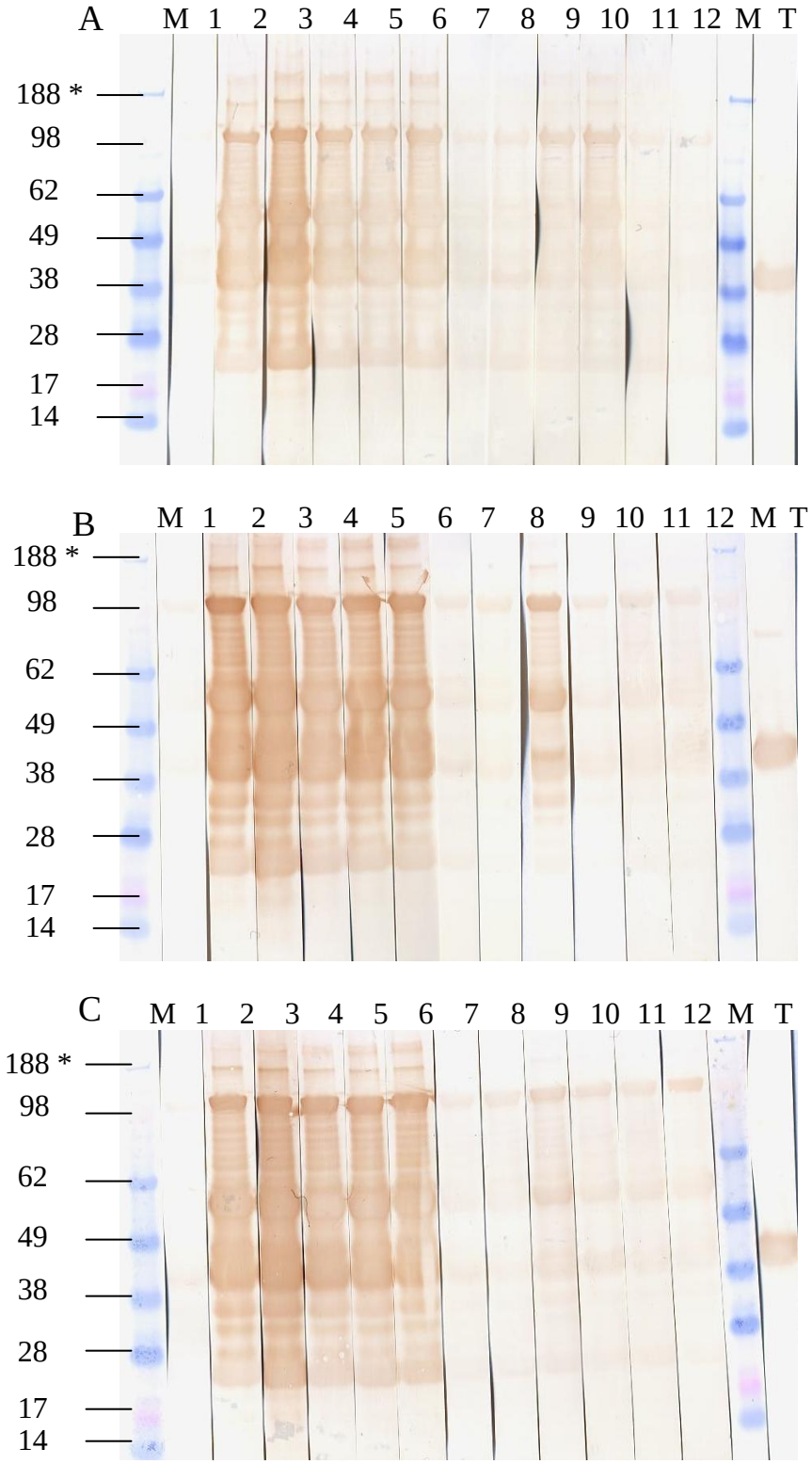


Figure 6.6. Western blots probing recombinant protein with antibodies raised against them and control antibodies for six time points throughout the experiment. Lane M: SeeBlue plus 2 molecular weight markers (Invitrogen). Lane T: tropomyosin protein probed with anti-tropomyosin as described in 6.2.5. The proteins loaded onto lanes 1 through 12 for all three blots were 2 µg of tropomyosin, paramyosin, cathepsin L. Panel A was probed with antibody from block H. Panel B was probed with antibody from block M. Panel C was probed with antibody from block L. Lanes 1 and 7: probed with antibody from week 10. Lanes 2 and 8: probed with antibody from week 16. Lanes 3 and 9: probed with antibody from week 18. Lanes 4 and 10: probed with antibody from week 22. Lanes 5 and 11: probed with antibody from week 26. Lanes 6 and 12: probed with antibody from week 30. * kDa

For the PBS-soluble protein fraction group, the density of DAB deposited on the Western blot prepared with the recombinant protein cocktail was normalised and the average of the three groups was plotted on a graph. Figure 6.7 is a graph of the normalised densitometry from the Western blot (figure 6.6) against time. The antibody densitometry rises from week 10 to week 18 where a difference can be clearly seen between the immunised and control groups. A t-test performed as described in 6.2.4, showed that there was a significant difference between the test recombinant and control groups for three of the time points. The *p* value on weeks 16 and 18 was less than 0.05 and on week 26 was less than 0.01.

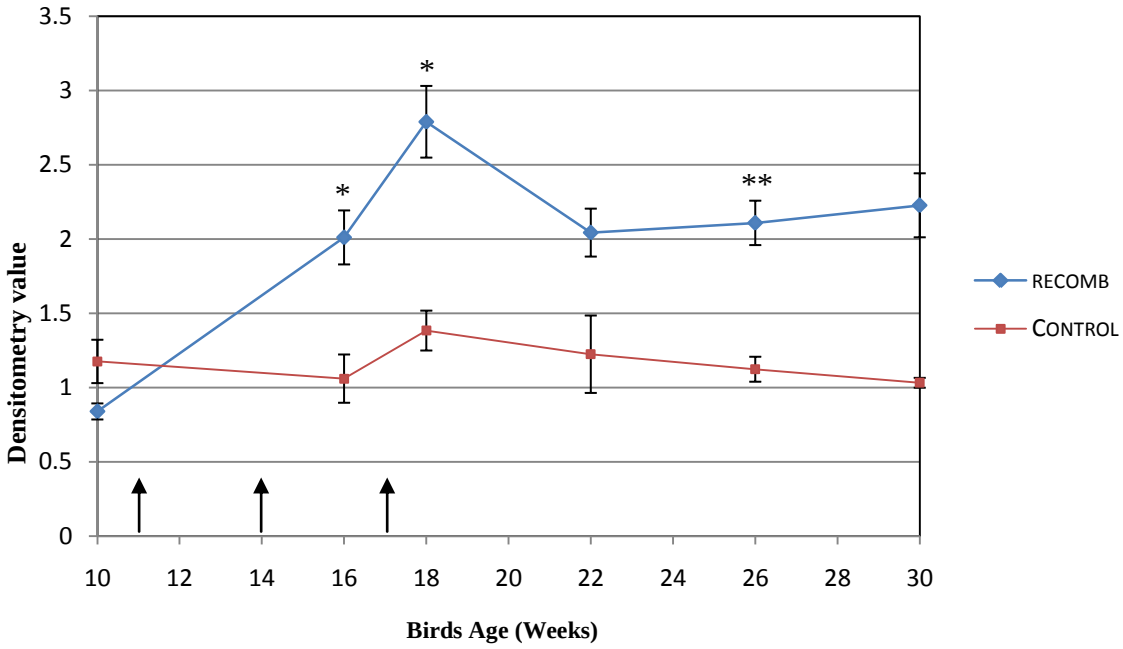


Figure 6.7. Graph showing the normalised intensities from the Western blots prepared with the recombinant protein cocktail. The densitometry value started at approximately 1. The antibodies in the test groups all increased to a maximum of almost 2.5. After the maximum at week 18, the antibody level for all three test groups stayed at an increased level compared to the controls. Throughout the experiment the antibody levels for the control groups did not vary significantly. Arrows indicate dates of immunisation. The error bars represent the standard error of the mean, n = 3. * indicates a *p* value of <0.05. ** indicates a *p* value of <0.01.

6.3.3 Monitoring mite numbers

ADAS MiteMonitor traps (ADAS limited, Oxon, UK) were used to monitor the population of the mites in the battery cages, which was used as an estimate of the total population of mites in the cage. The traps consists of two parts a holder that was attached to the cage and an insert that resembles corrugated cardboard that offer the mites a small dark places to hide. The insert, which contains the mites, can be removed and stored for mite collection.

Figure 6.8 is a graph that shows the average number of mites collected from the control, PBS-soluble protein and recombinant protein groups, as described in 6.2.4,

on weeks 19, 21, 23, 25, 27, 29 and 31. *D. gallinae* were first observed in the traps in week 21, where the total number collected was 26 mites from the PBS-soluble protein fraction groups, 20 mites from the recombinant protein cocktail group and nine mites from the control group. The number of mites collected from all groups increased sharply after week 27, following after the second challenge was administered. As can been there are more mites collected from the PBS-soluble protein fraction group and the recombinant cocktail group than the control group. There were no statistical differences between the groups.

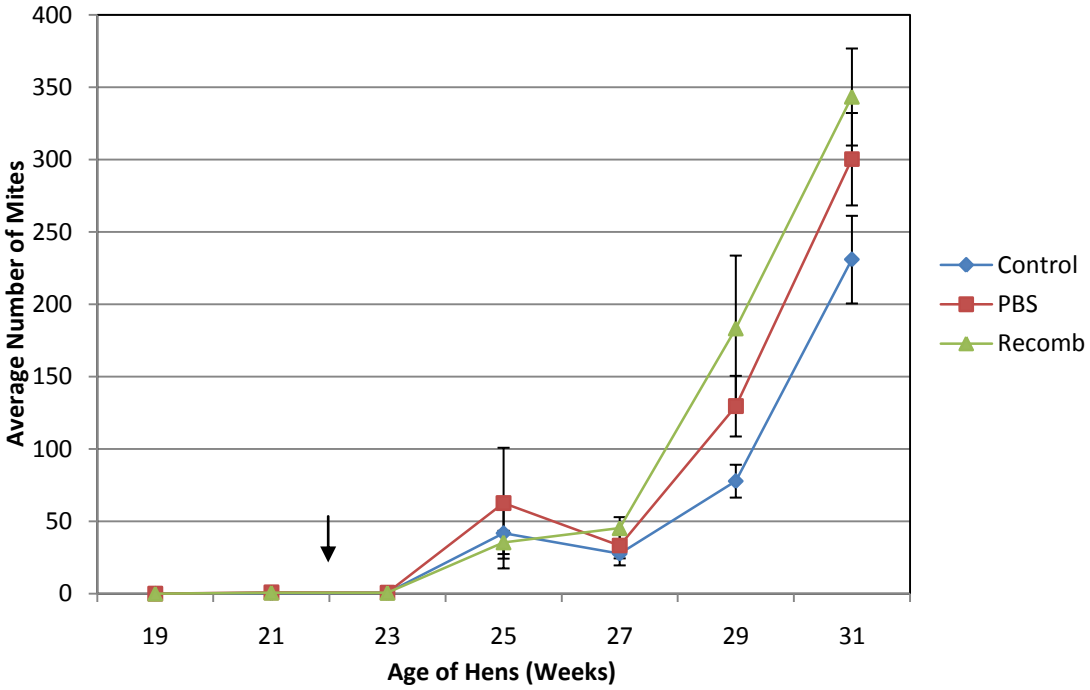


Figure 6.8. A graph showing the number of mites collected from all three groups during the course of the experiment. Each point on the graph represents the average count of 30 cages. The error bars represent the standard error of the mean, n = 3. The first challenge was added on week 19. The arrow represents the addition of the second mite challenge.

6.4 Discussion

The experiments described in this chapter were carried out to assess the capability of the PBS-soluble protein fraction and a cocktail of recombinant proteins to generate a protective immune response in laying hens against a challenge of *D. gallinae*.

Western blots were used to determine a semi-quantitative measure of antibody titre instead of enzyme linked immunosorbant assay (ELISA). The PBS-soluble protein fraction was a complex mix of proteins, which had molecular masses from less than 18 kDa to greater than 188 kDa. The visualisation of the immunoreactive proteins on Western blots gave a more informative analysis of the antibodies generated in the study. The Western blots for each cage block could be compared since a sample of recombinant tropomyosin probed with sera raised during the experiments in chapter 5 was used to normalise the data (figures 6.5 and 6.7). The Western blots showed that antibodies were generated against these proteins by the hens in each block of cages (figures 6.4 and 6.6). Even though antibodies were generated against both the PBS-soluble protein fraction and the recombinant protein cocktail, they were not effective in generating a protective immune response in the hens against *D. gallinae* over the period of study.

Antibodies raised against the PBS-soluble protein fraction detected protein across a range of molecular masses from 18 kDa to 188 kDa (figure 6.4). When the PBS-soluble protein fraction was probed with sera from the control group, proteins were detected at low molecular masses up to 38 kDa (figure 6.4). The normalised intensities showed that the intensity of the bands generated in the controls was not significantly lower than that of the bands generated when the test antibodies were used to probe the PBS-soluble protein fraction. The bands present on the control blot could represent antibodies generated against exposed antigens that were excreted or secreted from *D. gallinae* during the challenge, eliciting an antibody response. The data from the control group during this trial suggest that antibodies were generated against PBS-soluble mite proteins but did not lead to an increase in mite mortality in the control groups during the challenge stage of the experiment (figure 6.8).

The antibody responses generated to the PBS-soluble fraction were not equal between the replicates, as shown on the Western blots (figure 6.6) - the hens in the light group generated a higher antibody response. However, statistical analysis did not show a significant difference between the test and control groups when all three replicates were analysed. Bands can be seen on the Western blot probing the PBS-soluble protein fraction with the antibodies generated by the hens from the light group (figure 6.6, panel C), which was clearly greater than the antibody response generated by the medium and heavy hen groups. This could be due to a degradation of protein in the immunisation dose before injection of the heavy and medium groups. A more accurate measurement of antibody titre would be by ELISA. However, time restraints did not allow for an ELISA to be developed.

Bands were present on the Western blots in the control lanes, suggesting that there were antibodies in the control hens that recognised mite proteins with low molecular weights. A possible source of these immunoreactive proteins could be salivary proteins that were transferred to the hens whilst the mites were feeding. Unlike ticks, which can spend days feeding and have mechanisms to interrupt the host immune response (Ribeiro, 1987), *D. gallinae* feeds for a comparatively short period of time, approximately 1.5 hours per night. This timeframe would still allow significant transfer of antigens, although it is unlikely an effective protective immune response to these antigens would be mounted. Experiments would have to be performed that feed antibodies extracted from hens infested with *D. gallinae* to mites in the *in vitro* feeding assay, and then compare the mortality to that of mites fed blood from hens not infested with *D. gallinae*. Any significant differences between the mortality of the mites could be attributed to antibodies raised as a natural immunity to *D. gallinae.*

Although there is limited evidence to suggest that there is natural immunity in hens against *D. gallinae*, a study carried out in England showed no significant relationship between mite population size and antibody titres (Arkle et al., 2006). The Northern Fowl Mite (NFM) is another ectoparasite of hens. Unlike *D. gallinae*, it spends its

entire life cycle on the host, and a study has shown that the hens develop an antibody response to NFM. However, in that study the antibody response alone was not enough to affect the mites (Minnifield et al., 1993). Another study with NFM found that a local inflammatory response in the skin blocked access of the NFM mouthparts to the hens' blood vessels, firstly by an increase in epidermal thickness, which blocked the feeding of the nymph stage, and secondly by the formation of scabs by an exudate, which blocked the feeding of all mites (Owen et al., 2009). The NFM is always on the host, whereas *D. gallinae* is predominantly off the host where it reproduces, lays eggs and moults. The difference in life styles could be the major factor in why the hens develop some level of immunity to NFM but not to *D. gallinae*.

On the Western blots prepared with the recombinant proteins and probed with the test sera, intense bands can be seen at the molecular mass calculated for each of the proteins. Antibodies also bound to proteins with a molecular mass in between that of the recombinant proteins. These bands could represent breakdown products of the recombinant proteins that contain epitopes the antibodies recognise (figure 6.6). Bands were also present in the control blots to a varying degree, with some more intense bands indicating a stronger antibody response (figure 6.6). There was a significant difference between the intensities of the DAB deposited on the Western blots, and therefore between antibody titres, on weeks 16, 18 and 26. This difference in antibody titre could be due to the fact that the control hens were not exposed to these proteins during the challenge, since the proteins were probably not excreted or secreted while the mites were feeding. The lack of antibodies present in the control hens would suggest that the immune response could not be maintained naturally since the immune response would not be exposed to the proteins in this immunisation. This would mean the vaccination dose would have to prime the hen's immune system memory to ensure it produces antibody throughout its year-long laying life.

Even though a strong antibody response was generated against the recombinant protein cocktail, the antibodies did not appear to generate a protective immune

response against the hens *in vivo*. The hens were subjected to a challenge of *D. gallinae* mites at two points during the experiment. From the first mite challenge (week 19) approximately 1000 mites, were carefully purified from the cage debris they were collected in, sorted into challenge number by weight, and released into each cage. Very few mites were captured the first three times the traps were set out (weeks 19, 21 and 23 (figure 6.8)). This suggests that a challenge of 500 mites per bird was not enough to start a colony of *D. gallinae*, since very few mites found were even in the control cages. During a study where the population of *D. gallinae* was monitored in a free range laying unit, approximately 6 mites were collected per hen in the unit (Arkle et al., 2004), so a low count may have been expected. However, a second challenge (week 22) was added to the cages where the number of mites was much greater (at least 20 times greater than the first). The mites were added to the cages along with the cage debris they were collected in from the poultry shed, consisting of possibly faecal material, dead mites and hen skin. The debris contained a great many eggs so there was no way of knowing exactly how many mites were added to the experiment. The sudden increase in mite population between weeks 23 and 31 (figure 6.8) appears to have been directly due to the second addition of mites that may have overloaded the system. This conclusion was drawn because the average mite numbers started to increase three weeks after the second addition of mites, when an exponential increase in population size occurred. A study conducted in Denmark artificially infested ISA brown laying hens and monitored the mite population for 96 days and the behaviour of the infested hens in floor pens. A second challenge of mites was required since the initial challenge did not establish a viable colony. A conclusion drawn during that experiment was that a population of between 150,000 and 200,000 mites per hen would be required before clinical signs and health problems were observed in the hens (Kilpinen et al., 2005). In the current work, mite numbers grew exponentially five weeks after the second addition of mites, when there would have been antibodies present which recognised the proteins used for immunisation (figures 6.5 and 6.6). There was no reduction in the average number of mites in the immunised groups when compared to the control group, which would suggest that the immunisations did not have a protective element in the face of such a large challenge. However, the percentage of the mite population in

each of the cages that migrated into the traps was unknown. The detection method used, ADAS MiteMonitor traps, might not have been sensitive enough to measure the reduction in mite population. There was also a large variation in the number of mites collected within a single treatment group. The number of traps could probably have been reduced and left in the cages from one collection date to the next to allow the mites to find and then utilise the traps. For monitoring of mite population, a study conducted in Sweden left traps in place for the duration of the experiment, changing them once a week (Chirico & Tauson, 2002).

The challenge mites had not been conditioned prior to addition into the study. This meant that a proportion of the mites had recently taken a bloodmeal from unvaccinated hens with which they could moult, reproduce or lay eggs before they had to feed again. This fed mite population would have then increased immediately after the massive second challenge without being exposed to the hen's primed immune system. If the mites had been starved they would have had to feed as soon as they were able in order to moult, reproduce and lay eggs. This would have brought them directly into contact with the hen's immune system, and a more accurate test of the immune response generated against the injected proteins may have been made.

At the end of the experiment, the number of mites trapped was counted. As discussed in chapter 3, when mites have recently fed they appear bright red in colour, which darkens to almost black after a few days. If the levels of mites feeding and how recently they had fed had been scored, a more accurate picture of what was happening in the cages could have been gathered. A week after the second challenge was added, the number of mites increased, however the number of mites at each life cycle stage was not recorded, so these could have been all larval or nymph stages that had not fed.

During the experiment, other measurements were taken by colleagues at SAC Auchincruive, such as haematology, feed intake, egg production, external and internal egg quality, feather score, behaviour and mortality. There was no statistically significant effect of the any of these factors except haematology. That these factors

were not affected by the injected proteins was a positive outcome since the poultry would still have to perform at peak efficiency during their laying life or any potential vaccine would not be commercially viable.

There were seven haematological tests carried out on the blood from the hens: red blood cell count, haemoglobin, haematocrit, mean cell volume, mean corpuscular haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and white blood cell counts. Linear mixed models (LMM) were fitted using residual maximum likelihood (REML) to all seven measurements. There was no statistically significant difference in five of the tests. However, there was a statistically significant difference in the means of the MCHC and MCH measurements when compared to the controls at week 32, where the means were lower in both test groups than in the control. The MCHC is a measurement of the concentration of haemoglobin for a given volume of red blood cells and the MCH is a measurement of the mean amount of haemoglobin in a red blood cell. The drop in haemoglobin concentration did not appear to affect the performance of the birds in any way as there was no statistical difference between the three groups for egg production and feather score.

A generalised linear mixed model (GLMM) was fitted using REML to the counts of the different activities a bird could perform during each monitoring period. There was marginally more feather pecking by the control hens than hens injected with either the PBS-soluble protein fraction or the recombinant protein cocktail ($p = 0.038$). The injected proteins could have resulted in a reduction of the number of mites on the hens in the test groups since they did not appear to peck their feathers as much. The control hens might have been more heavily parasitized than the immunised hens, though there was no evidence for this from the mite trap data.

This experiment did not show clear evidence that the PBS-soluble protein fraction or the recombinant protein cocktail generated a protective antibody response. However, as discussed, the experimental system may have been overloaded with mites after the second mite challenge and the duration of study was truncated because of this. If this experiment was to be undertaken again, the number of mites initially added to the

system would have to be increased and properly conditioned, which would ensure that the mites added to the system would have to feed from the hens to survive and propagate. The simplest method to achieve this would be to use a set of cages that were already infested with *D. gallinae* or for the cages to be specifically infested shortly before the hens were introduced. Also, a more sensitive method for detecting changes in the mite population would be beneficial for future studies since the MiteMonitor traps were only an indirect measure of parasite numbers.

Chapter 7:

General Discussion

Dermanyssus gallinae is an haematophagous ectoparasite that parasitizes poultry. A population of mites can increase exponentially over only a few weeks in optimum conditions. When hens are heavily infested by *D. gallinae* they suffer from anaemia and severe irritation, which can lead to restlessness, feather pecking and death (of hens). Currently, the most successful control of *D. gallinae* is based on spraying chemical acaracides and cleaning empty cages before restocking (Chauve, 1998). The use of chemical control is unsustainable due to the emergence of acaricide resistance in mites (Marangi et al., 2009a; Zeman & Zelezny, 1985) and contamination of the environment and food products with harmful chemicals (Hamscher et al., 2003). At present the only acaricide spray licensed for use in the UK, when cages are stocked, is ByeMite® (Animal Health, Bayer Health Care) (Meyer-Kuhling et al., 2007). Alternative control methods that have no adverse effects on poultry or the environment need to be identified. This study was performed to identify protein antigens that could be used as a vaccine against *D. gallinae*.

Both pragmatic and rational approaches were employed during this study to identify proteins from *D. gallinae* that could be used in a vaccine. The pragmatic approach was used to identify potential vaccine candidates. Hens were immunised with fractions of *D. gallinae* and mites were fed the resultant antibodies in the *in vitro* feeding assay. The increase in mortality of the mites was then measured (Wright et al., 2009). The results indicated that the PBS-soluble protein fraction generated antibodies that increased the mites' mortality to the greatest extent (chapter 3). This approach has been successful in identifying the protein Bm86, which is found in the membrane of gut digest cells of the cattle tick *Rhipicephalus microplus* (Willadsen et al., 1988; Willadsen et al., 1989). Bm86 is the antigenic element of TickGARD™ and Gavac™ (Revetmex, Mexico City) (Willadsen et al., 1995), which have been the only commercially available vaccines on the market against ectoparasites. When ticks feed on vaccinated animals, antibodies specific to Bm86 bind to the protein in the gut of the tick and the digest cells are destroyed. The mechanism of this destruction has been investigated, and evidence would suggest that anti-Bm86 antibodies bind to the gut membrane-bound protein, causing antibody-mediated

products, such as complement, to lyse the cells using the membrane attack complex. Lysis of the digest cells allows bovine blood to leak into the tick haemolymph. The observed effects on the tick are low weight gain during engorgement, a reduction in the number of eggs laid and death of the tick (Kemp et al., 1989). In the mammalian immune system, the Fc region of IgG bound to antigen activates immunological processes such as the complement cascade, as described in chapter 1. Since avian IgY has a different heavy chain, and therefore a different Fc region, it cannot activate the complement cascade in hens (Cova, 2005). Although IgY does not activate the hen's complement cascade in the same manner as mammalian IgG, a homologue of the mammalian C1q protein, which is part of the antibody binding C1 complex, has been found in hen blood (Yonemasu & Sasaki, 1986). Therefore the complement system may damage the gut of *D. gallinae* in a similar way to the damage seen in *R. microplus*. TickGARD™ was very effective at reducing the tick population on cattle. During a trial in Australia, there was a 56 % reduction in tick numbers after a single generation and a reduction of 72 % in reproductive capacity (Jonsson et al., 2000). Although the vaccine could not generate a long lasting immunity, in a trial where 35 cattle were injected with Gavac™ and then challenged with *R. microplus*, after 6 weeks the number of ticks had built up to an average of 20 per animal (Garcia-Garcia et al., 2000). Another vaccination trial was carried out where cattle were injected with either TickGARD™ or Gavac™ at weeks zero and four. Measurements of the antibody response showed a maximum titre was reached by week six, after which the titre dropped steadily during the 11 week trial (Andreotti, 2006). To maintain the circulating antibody titre, doses of the vaccine had to be given throughout the year. A commercially viable vaccine for *D. gallinae* (based on hidden antigens) would have to elicit an antibody response that had an enhanced titre throughout the hen's year-long laying life. Although proteins similar to Bm86 have not been identified in *D. gallinae*, the proteins that line the parasite's gut could be a possible source of vaccine candidates. Proteins located in the gut are "hidden" from the host, so any immune response would have to be initiated and maintained by repeated exposure to the antigen(s) by immunisations, rather than during infestation. Despite the fact that the TickGARD™ vaccine was very effective, it was withdrawn from sale by Pfizer. However, since the Bm86 based vaccines were introduced to the

market, several other single antigens have been discovered that offer protection against ticks, as well as some other parasitic species. Although there has been no evidence of a homologue of Bm86 in *D. gallinae*, an immunisation trial has been performed that involved injecting hens with Gavac™ and a recombinant form of subolesin from the tick *Amblyomma hebraeum*. In these vaccinated hens, antibodies against Bm86 but not against subolesin were detected on Western blots. Blood from immunised hens was fed to mites in an *in vitro* feeding assay. The mortality of the mites fed blood from Bm86 immunised hens ranged from 0 % to 84.6 %, which was not significantly different from the mortality of the mites fed control blood. The mortality of the mites fed blood from hens immunised with subolesin ranged from 25 % to 78.6 % and were statistically different from the control group (Harrington et al., 2009a). Subolesin has the potential to be a vaccine candidate and more of the potential vaccine antigenic candidates from different parasitic species need to be investigated. One candidate could be Bm95, a homologue of Bm86 in an Argentinean strain of *R. microplus*, strain A, which is resistant to Gavac™. The recombinant form of the protein offered 58 % efficacy against strain A (Garcia-Garcia et al., 2000). Another candidate would be the salivary protein 64P, truncated recombinant forms of which were used to immunise guinea pigs against *R. sanguineus*, resulting in an 80 % mortality of the ticks (Trimnell et al., 2005). Cysteine proteases might also be potential vaccine candidates, as they have been found to play a key role in the feeding mechanisms of the fluke *F. hepatica*. The recombinant form of the fluke cysteine protease cathepsin L was used to vaccinate mice, which were then challenged with fluke parasites. The immune response generated reduced the parasitic burden by up to 80 % (Kesik et al., 2007). Since the success of Bm86 has not been repeated, and no other single antigen has been identified that has as high a degree of efficacy (Willadsen, 2008). Future vaccines may have to be composed of multiple antigens in a cocktail that together generate a protective immune response capable of reducing the parasite population. A further possibility would be to target different life stages of the parasite with either a combination of proteins common to all stages or different proteins that target each of the blood feeding stages (Tellam et al., 2002).

A vaccine, either with a single antigen or a cocktail of antigens, would probably have to be used in combination with acaracides to reduce the levels of *D. gallinae* infestation of poultry units to a manageable level. This would reduce the use of potentially harmful chemicals (de la Fuente et al., 2007) and target mites that have developed resistance to acaracides. This approach has been successful in Cuba where the use of Gavac™ in combination with acaracides reduced the number of chemical treatments by 60 % (de la Fuente et al., 1999). The combination control strategy could be implemented at the introduction of a new flock to a poultry house. All the hens would be vaccinated and the cages sprayed with a chemical to initially reduce the mite population. After this initial combined regime a limited number of sprayed chemical treatments throughout the laying period of the flock could restrict the build up of mite numbers. The reduction in chemical treatments could slow the development of resistance in *D. gallinae* and reduce costs to the farmer. This has been shown in *R. microplus*, where Gavac™ was used to reduce a population of ticks resistant to amidine acaracides to zero after 83 days (Redondo et al., 1999). Using a vaccine and chemicals in combination to control *D. gallinae* could mean that the vaccine would not have to be as effective as TickGARD™. As stated previously, *D. gallinae* is a source of great economic loss to the poultry farmer through blood spots on the eggs and reduced performance of the parasitized hens due to blood loss and irritation. Feeding antibodies to mites raised against the PBS-soluble protein fraction reduces the population by 39 %. In practice, a reduction in population by this amount would mean there would be less blood-spotting and increased productivity of the hens, thereby increasing profit margins.

As described in the general introduction, the development of resistance to chemical compounds is an increasing problem (Beugnet et al., 1997). Vaccination would circumvent this resistance issue, and, arguably there is little likelihood of the mite becoming non-responsive to the vaccine, since most proteins have multiple epitopes to which an antibody can bind. For a parasite to develop resistance, the native protein would have to have multiple mutations in the DNA encoding these epitopes, changing the amino acid sequence and configuration but without changing function. Such a mutation would take multiple generations to occur, and result in inactive

proteins and possibly lethal changes. The development of resistance to a vaccine based on targeting “hidden” or “exposed” antigens is probably less likely than the development of resistance to chemicals, which can occur with single nucleotide polymorphisms (SNPs). For example, resistance against the benzimidasole class of anthelmintic, which interrupts the tubulin-microtubule equilibrium in the cell cytoskeleton in gastrointestinal nematodes, is thought to be caused by up to three SNPs that alter the amino acid sequence of the protein β-tubulin at bases 167, 198 and 200. These changes to the β-tubulin amino acid sequence do not allow the drug to bind and disrupt the tubulin-microtubules equilibrium (Von Samson-Himmelstjerna et al., 2007). The antibody raised against a vaccine may bind to an external epitope that is under no selective pressure to mutate, so the SNP would be less likely to occur. However, in *D. gallinae* there could be significant differences between strains. An Argentinean strain of *R. microplus*, strain A, has been shown to be resistant to the vaccine Gavac™ (Garcia-Garcia et al., 1999). The homologous protein to Bm86 in this strain of tick, which was called Bm95, had up to 21 amino acid differences to Bm86 (Garcia-Garcia et al., 2000). Strain differences and SNPs may be present in potential vaccine candidate genes from *D. gallinae*, since differences have been found in the cytochrome oxidase I gene in mites from populations in European countries (Marangi et al., 2009b). A potential vaccine antigen would have to be tested against different populations of mites to ensure its efficacy and potential to be used against *D. gallinae* infestations in the UK, in Europe and, ideally, world-wide.

In the present study, the rational approach to vaccine candidate identification was also employed, where genetic sequencing approaches were used to identify vaccine candidates, homologues of which have been used successfully to protect against other pathogenic species. Expressed sequence tag (EST) databases were generated that could be mined for vaccine candidates. Two databases were generated to produce over 13,000 contigs using material derived from total RNA (chapter 4). A third EST database was generated using a suppression subtractive hybridisation (SSH) library to identify genes that were differentially expressed between fed and starved mite populations. The fourth dataset was generated using Illumina Solexa

sequencing to quantitatively analyse the differentially expressed genes between fed and starved mites. There were genes in the SSH EST database that were shown to be up-regulated in the Illumina Solexa dataset. All four datasets identified proteins that could be possible vaccine candidates against *D. gallinae*.

Two such proteins are paramyosin and tropomyosin, which are proteins of the cellular cytoskeleton (Maroto et al., 1995; Perry, 1985). These proteins have been shown to generate an anti-parasitic immune response in endoparasites. A native form of paramyosin has been successfully employed as a vaccine that reduces the burden of *Schistosoma japonicum* in mice by up to 86 % (Ramirez et al., 1996). When expressed as a recombinant protein it reduced the burden of *S. japonicum* in mice by up to 39.9 % (Zhang et al., 2006). Native and recombinant versions of tropomyosin have been used to reduce the burden of *Acanthocheilonema viteae*, a filarial nematode, in jirds by 64.4 % and 35 % respectively (Hartmann et al., 1997; Hartmann et al., 2006). Paramyosin has also previously been identified as a potential vaccine candidate against *Psoroptes ovis*, the sheep scab mite, (Nisbet et al., 2006b) and tropomyosin has been identified in *D. gallinae* (Nisbet et al., 2006a). Paramyosin, which was partially sequenced by Roche 454 sequencing, and tropomyosin, which had been sequenced and cloned previously (Nisbet et al., 2006a), were expressed and used in an immunisation study to raise antibodies, which were tested using the *in vitro* feeding assay. This experiment demonstrated that the antibodies generated caused a mite mortality of 33 % in paramyosin groups and 28 % in tropomyosin groups, compared to a mortality of 9 % in the control group (chapter 5). Although antibodies to these proteins had anti-mite properties when tested in the *in vitro* feeding assay individually, time did not allow the investigation of whether a combination of these antibodies would have resulted in an enhanced mortality. Clearly, it would be important to determine whether the average of 30 % mortality in the mite population with each protein could be increased with a combination of antibodies, as has been previously suggested during studies for developing vaccines (Willadsen, 2008). An average mortality of 30 % was also achieved with the PBS-soluble protein fraction, which was a complex mix of proteins. Future studies are

required to define the active antigen in the complex mixture, and to establish whether multiple protein antigens will enhance the efficacy of a vaccine.

An *in vivo* study was performed in which hens were injected with a recombinant protein cocktail, which included paramyosin and tropomyosin along with histamine release factor, cathepsin L and cathepsin D, all of which were identified in the EST database generated using Roche 454 sequencing technology. These were categorised in the post-translational modification, protein turnover or chaperones group (chapter 4). The hens were then challenged with live *D. gallinae*. The PBS-soluble protein fraction was also used to immunise hens (chapter 6). The *in vivo* experiment did not provide a definitive answer as to whether the immune response generated by either the recombinant protein cocktail or the PBS-soluble protein fraction was protective against a mite challenge. The mites that were used in the challenge were freshly collected from a commercial poultry farm and introduced to the hens a day later. Clearly the initial mite challenge of approximately 500 mites per hen was insufficient, in the time allowed, to establish a stable colony. So a second challenge of mites, which was greater than 10,000 mites per cage, was added to the experiment. There are two critical factors that would need investigation before future immunisation studies are performed. These are 1) the number of mites introduced to the cages would have to be optimised and 2) the mites added could be conditioned before challenge for at least a week to allow the bloodmeal from hens on the commercial farm to be digested. As discussed above, the number of mites used to start a challenge is crucial, as too many mites could overload the system and cause welfare issues for the experimental hens, whereas too few mites would result in the challenge not being established. The conditioning of mites allows the bloodmeal to be digested and for the next stage of the life cycle to be completed, whether that is moulting, reproducing or laying eggs. If the majority of the mites used in the challenge were female adults that contain a clutch of eggs, then the number of mites in the challenge would be much higher than estimated. If the mites were conditioned they would have to feed on the hens to complete their life cycle. The population of mites feeding on the control hens would increase as normal, however the mites

feeding on the immunised hens would decrease due to the antibodies generated against the injected antigen.

There are several important factors which are involved in the development of a successful vaccine. These include not only the most effective antigen target, but the dose, route of inoculation, the adjuvant employed and the frequency of vaccination. In addition, a vaccine produced to combat *D. gallinae* would have to be applied to the flock using a system that can be easily managed. Most poultry farms have a strict vaccination schedule that starts soon after the chick is hatched. The hens are vaccinated against various diseases before they are housed. These included a vaccine against Marek's Disease, which is injected subcutaneously in the back of the neck (Baigent et al., 2006), and vaccines against Newcastle Disease and Infectious Bursal Disease (Sharma, 1999), which can be administered in either the water supply or as a whole flock spray. It may be possible to combine a vaccine against *D. gallinae* with one of these other vaccines to reduce the stress and expense caused by another injection. The use of non-invasive application strategies, such as introducing the vaccine to the water supply, may be of use. However, these spray and water-borne applications are most effective for live vaccines, and immunisations for inert protein antigens are likely to require injection. Stability of the vaccine is of paramount importance, and factors such as resistance to enzymic digestion may all be relevant. For example, if the vaccine consists of a cocktail of antigens and one of them is the active form of a digestive enzyme, the other proteins may be digested during storage, potentially rendering the vaccine ineffective.

Further work to develop a vaccine against *D. gallinae* is required. A second challenge trial would be necessary to properly assess the potential of the PBS-soluble protein fraction (from *D. gallinae*) as a vaccine. In any further study, the number of mites used to populate the cages would have to be closely regulated. The mites should be partially conditioned before being introduced to the cages to ensure that they take a bloodmeal from the hens in the study. The immunoreactive proteins from this study should then be identified, e.g. using 2D Western blots and MALDI-ToF analysis, so that recombinant forms of the proteins can be expressed and used in

immunisations trials. The antibodies generated during future trials can be tested in the *in vitro* feeding assay before challenge studies using large numbers of hens. As discussed previously, there are a number of issues that require addressing, which are the determination of the relationship between IgY titre and anti-mite efficacy, the number of injections and the timing of these to optimise protection, and the adjuvant required. These issues will all need to be defined before any vaccine candidate is licensed for production. Moreover, also of high importance will be the demonstration that the effector antibodies are safe for human consumption. The development of TickGARD™ took 13 years, which was considered a short period for a novel vaccine. It went through four stages demonstration of feasibility in the laboratory, demonstration of feasibility in field trials, proof of efficacy, safely and applicability and integration of product into commercial market (Willadsen et al., 1995). Registration of a vaccine in the EU has to be ratified by the European Medicines Evaluation Agency (Meeusen et al., 2007). Although antibodies are inactivated by acid in the stomach or degraded by proteases in the gut, their lack of reactivity with similar or cross-reactive epitopes in human tissues would need to be demonstrated.

This thesis described the initial studies towards the production of a vaccine against the poultry red mite, *D. gallinae*. The pragmatic approach employed in this study suggested that the PBS-soluble antigen from *D. gallinae* was capable of increasing the mortality of mites in an *in vitro* assay. The rational approach was also employed, and by use of genetic sequencing tools produced a database of thousands of contigs and another database containing differentially expressed genes between populations of fed and starved *D. gallinae* mites. Two previously identified vaccine candidates, tropomyosin and paramyosin, that had an effect against other parasitic species, were shown to elicit the production of antibodies in hens that increased the mortality of mites in the *in vitro* feeding assay. An *in vivo* trial was performed in which the PBS-soluble protein fraction, identified using the pragmatic approach, and five recombinant proteins, which included paramyosin and tropomyosin, identified using a rational approach, were injected into hens that were subsequently challenged with live *D. gallinae*. As described earlier, the experiment was not successful in producing reliable results, therefore alterations to the experimental design would have to be

implemented. A successful vaccine, combined with chemical control methods for maximum efficiency, would have a direct and lasting effect on the poultry industry, in particular for egg production. Quality of life and productivity of laying hens would be improved, resulting in a reduction in profit losses for farmers.

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Appendix 1:

Table of contigs generated from SSH library

Contig	Length	Nos. of EST	Contig identity/Putative function	*P* value	Species	Database
DBC003	354	3	Similar to CG30375-PA Peptidase_S1_S6	2E-07	*Drosophila melanogaster*	KEGG
DBC027	391	1	Similar to spicule matrix protein SM29	8E-04	*Strongylocentrotus purpuratus*	KEGG
DBC032	321	1	Eukaryotic translation initiation factor 3	2E-20	*Xenopus tropicalis*	KEGG
DBC037	352	1	K05868 cyclin B	8E-15	*Xenopus laevis*	KEGG
DBC039	643	1	Hypothetical protein	3E-07	*Saccharum officinarum*	KEGG
DBC048	291	1	3-oxoacid CoA-transferase subunit A	2E-14	*Fugu rubripes*	KEGG
DBC050	710	1	Similar to CG1746-PA ATPase C chain family, isoform A	1E-05	*D.melanogaster*	KEGG
DBC052	495	18	Hypothetical protein	8E-03	*Arabidopsis thaliana*	KEGG
DBC063	592	1	zgc:136473	8E-19	*Danio rerio*	KEGG
DBC066	548	1	tRNA (cytosine-5-)-methyltransferase	6E-32	*Oryzias latipes*	KEGG
DBC067	603	5	Hypothetical protein	7E-04	*Plasmodium falciparum*	KEGG
DBC074	391	1	Similar to spicule matrix protein SM29	2E-03	*Strongylocentrotus purpuratus*	KEGG
DBC077	359	9	NEWSINFRUG00000126286	9E-03	*P. falciparum*	KEGG
DBC078	295	1	Similar to 2300006N05Rik protein keratin high-sulfur matrix protein IIIA	5E-04	*mouse*	KEGG
DBC087	320	1	Protein tyrosine phosphatase, receptor type, D	3E-05	*Oryzias latipes*	KEGG
DBC094	652	1	Methylmalonyl-CoA epimerase	6E-40	*D. melanogaster*	KEGG
DBC099	483	3	NPC2; Niemann-Pick disease, type C2	5E-07	*Sus scrofa*	KEGG
DBC107	306	6	Similar to CG4769-PA	1E-29	*D. melanogaster*	KEGG
DBC110	648	4	Hypothetical protein	9E-03	*Homo sapiens*	KEGG
DBC111	367	3	Hypothetical protein	3E-10	*Malassezia globosa*	KEGG
DBC113	457	1	Predicted protein; K08776 puromycin-sensitive aminopeptidase	1E-12	*Nematostella vectensis*	KEGG
DBC116	368	1	Mannose-6-phosphate protein p76	5E-16	*Bos taurus*	KEGG
DBC117	242	1	Similar to aldehyde dehydrogenase 7 family, member A1	3E-34	*Tribolium castaneum*	KEGG
DBC119	270	6	Similar to CG10686-PA Cellular component: transcription factor complex	1E-10	*D. melanogaster*	KEGG
DBC134	307	3	Cathepsin D	9E-22	*macaca-mulatta*	KEGG

Contig	Length	Nos. of EST	Contig identity/Putative function	*P* value	Species	Database
DBC136	347	1	Hypothetical protein; Interacting selectively with any nucleic acid.	7E-03	*Monosiga brevicollis*	KEGG
DBC138	443	1	Annexin x	1E-16	*Aedes_aegypti*	KEGG
DBC142	254	1	GE24263-RA	3E-05	*D. yakuba*	KEGG
DBC144	405	2	U1 small nuclear ribonucleoprotein A U2 B	2E-06	*Anopheles_Apis*	KEGG
DBC151	470	3	GI15535-RA	5E-03	*D. mojavensis*	KEGG
DBC159	257	1	Similar to MGC80934 protein coatomer protein complex, subunit beta 1	2E-22	*Xenopus laevis*	KEGG
DBC160	469	1	GA20895 gene product from transcript GA20895-RA	7E-11	*D. pseudoobscura*	KEGG
DBC163	418	3	Rab proteins geranylgeranyltransferase component A 1	8E-07	*Homo sapiens*	KEGG
DBC164	520	1	Sphingomyelin phosphodiesterase 1	4E-14	*Mus musculus*	KEGG
DBC182	731	1	Hypothetical protein	8E-05	*Podospora anserina*	KEGG
DBC184	452	1	GF19153-RA	6E-04	*Drosophila ananassae*	KEGG
DBC190	490	1	Hypothetical; solute carrier family 38, member 8	2E-17	*Strongylocentrotus purpuratus*	KEGG
DBC204	338	1	K02132 F-type H+-transporting ATPase alpha chain	7E-14	*A. gambiae*	KEGG
DBC210	224	1	GF10431-RA	1E-02		KEGG
DBC214	449	1	Similar to vacuolar protein sorting 35	4E-32	*Tribolium castaneum*	KEGG
DBC219	269	1	GH17039-RA	3E-05	*D. grimshawi*	KEGG
DBC223	311	1	K02920 large subunit ribosomal protein L36e	5E-28	*C.merolae*	KEGG
DBC229	279	1	NEMVEDRAFT_v1g244189; predicted protein	2E-21	*Nematostella vectensis*	KEGG
DBC237	326	1	Hypothetical protein	5E-03	*Paramecium tetraurelia*	KEGG
DBC238	274	1	Hypothetical protein	7E-11	*Homo sapiens*	KEGG
DBC239	215	1	Hypothetical LOC579671 ; K01870 isoleucyl-tRNA synthetase	3E-19	*C.merolae*	KEGG
DBC240	512	1	DeliriumA, putative	2E-04	*Tetrahymena thermophila*	KEGG
DBC242	403	1	Cathepsin D ; K01379	6E-20	*Macaca mulatta*	KEGG
DBC244	428	1	Similar to lethal 35Di	2E-20	*Tribolium castaneum*	KEGG
DBC249	671	1	Cathepsin L-like precursor; K01365 cathepsin L	2E-16	*M.musculus*	KEGG

Contig	Length	Nos. of EST	Contig identity/Putative function	*P* value	Species	Database
DBC250	379	1	Isoform A amylo-1,6-glucosidase	4E-17	*Tribolium castaneum*	KEGG
DBC254	751	1	SET domain and mariner transposase fusion gene	3E-04	*Pan troglodytes*	KEGG
DBC261	520	1	GE18641-RA	4E-10	*Drosophila*	KEGG
DBC263	419	1	Acyl-CoA synthetase long-chain fatty-acid-CoA ligase	1E-08	*Acinetobacter*	KEGG
DBC264	727	1	Hypothetical protein	9E-03		KEGG
DBC266	659	1	Predicted protein	1E-07	*Nematostella vectensis*	KEGG
DBC270	462	1	GI18840-RA	6E-26	*D. mojavensis*	KEGG
DBC271	218	1	Carboxylesterase	2E-23	*Xanthomonas campestris*	KEGG
DBC275	356	1	Pold2-prov protein; DNA polymerase delta subunit 2	6E-17	*Brugia malayi*	KEGG
DBC276	536	1	Serine/threonine protein kinase	5E-03	*Frankia*	KEGG
DBC281	507	1	Similar to CG13625-PA	5E-17	*D. melanogaster*	KEGG
DBC283	435	1	Alpha-L-fucosidase	6E-55	*Bombyx mori*	KEGG
DBC286	535	1	Cysteine proteinase, putative	1E-25	*G.gallus*	KEGG
DBC288	452	1	BMP binding endothelial regulator	9E-03	*Bos taurus*	KEGG
DBC290	203	1	Adenosylhomocysteinase	2E-06	*Caenorhabditis elegans*	KEGG
DBC295	449	1	Similar to proteasome 20S proteasome subunit alpha 1	2E-10	*Tribolium castaneum*	KEGG
DBC296	809	3	Dihydrolipoamide succinyltransferase	1E-07	*Caenorhabditis elegans*	KEGG
DBC297	318	1	GJ11095-RA	1E-18	*D. melanogaster*	KEGG
DBC298	316	1	Similar to Protein C14orf166	2E-09	*Homo sapiens*	KEGG
DBC301	333	1	Carboxypeptidase A4	2E-07	*Mus musculus*	KEGG
DBC306	256	1	Hypothetical protein cathepsin L	2E-03	*Mus musculus*	KEGG
DBC308	363	1	Alpha-glucosidase	4E-06	*D. melanogaster*	KEGG
DBC322	315	1	Protein disulfide isomerase family A, member 3	3E-06	*D. melanogaster*	KEGG
DBC323	822	1	Glutathione S-transferase M2	1E-19	*Homo sapiens*	KEGG
DBC324	228		Senescence-associated protein	2E-15	*Brugia malayi*	KEGG

Contig	Length	Nos. of EST	Contig identity/Putative function	*P* value	Species	Database
DBC328	471	1	Hexosaminidase B ; K01207 beta-N-acetylhexosaminidase	5E-35	*Mus musculus*	KEGG
DBC001	356	1	Similar to hydroxyacyl-coenzyme A dehydrogenase	5E-28	*Tribolium castaneum*	NCBI nr
DBC005	508	21	GP80 precursor	9E-13	*Boophilus microplus*	NCBI nr
DBC019	822	12	Vitellogenin	9E-24	*Dermacentor variabilis*	NCBI nr
DBC021	722	2	Mariner transposase	5E-41	*Homo sapiens*	NCBI nr
DBC022	282	1	ATP synthase D chain	4E-13	*Ornithodoros parkeri*	NCBI nr
DBC047	435	2	Cathepsin D-like aspartic protease	4E-40	*Opisthorchis viverrini*	NCBI nr
DBC057	423	2	Similar to CG13310-PA	3E-28	*Apis mellifera*	NCBI nr
DBC059	287	1	Protein disulfide isomerase-1	4E-32	*Haemaphysalis longicornis*	NCBI nr
DBC075	675	22	Vitellogenin structural genes (yolk protein genes) protein 2, isoform a	1E-11	*Caenorhabditis elegans*	NCBI nr
DBC086	433	4	Vitellogenin	2E-12	*Dermacentor variabilis*	NCBI nr
DBC090	463	1	Aspartic protease	5E-20	*Haemaphysalis longicornis*	NCBI nr
DBC100	543	1	Ribosomal protein L30	3E-11	*Ornithodoros parkeri*	NCBI nr
DBC105	472	4	RNA (guanine-9-)-methyltransferase domain-containing protein 1 homolog	1E-20	*Tribolium castaneum*	NCBI nr
DBC106	660	4	Vitellogenin	4E-33	*Dermacentor variabilis*	NCBI nr
DBC108	573	3	Vitellogenin C2	6E-08	*Culex pipiens quinquefasciatus*	NCBI nr
DBC112	562	9	Similar to CG13313-PA (Spermadhesin, CUB domain)	8E-17	*Apis mellifera*	NCBI nr
DBC115	778	2	GP80 precursor	4E-12	*Boophilus microplus*	NCBI nr
DBC121	242	2	Similar to granulin	2E-09	*Strongylocentrotus purpuratus*	NCBI nr
DBC129	384	1	Arginine kinase	2E-51	*Dermatophagoides farinae*	NCBI nr
DBC132	710	3	Similar to mitochondrial ribosomal protein L53 CG30481-PA	3E-14	*Tribolium castaneum*	NCBI nr
DBC139	216	1	Similar to ENSANGP00000030885	3E-08	*Nasonia vitripennis*	NCBI nr
DBC143	224	1	Hypothetical protein CBG14484 (Protein disulphide isomerase)	4E-27	*Caenorhabditis briggsae*	NCBI nr
DBC146	337	1	p27BBP/eIF6 (LOC398731 protein)	3E-48	*Xenopus laevis*	NCBI nr
DBC148	461	1	prp8 (similar to CG8877-PA)	6E-82	*Apis mellifera*	NCBI nr

Contig	Length	Nos. of EST	Contig identity/Putative function	*P* value	Species	Database
DBC165	321	2	Similar to 2-oxoglutarate dehydrogenase	8E-26	*Nasonia vitripennis*	NCBI nr
DBC179	567	1	Calreticulin	8E-79	*Ixodes jellisoni*	NCBI nr
DBC180	610	2	Protein disulfide isomerase-2	1E-10	*Haemaphysalis longicornis*	NCBI nr
DBC195	506	1	Vitellogenin	3E-22	*Dermacentor variabilis*	NCBI nr
DBC213	452	3	Nucleoside diphosphate kinase	6E-56	*Ornithodoros parkeri*	NCBI nr
DBC217	344	1	Branched chain keto acid dehydrogenase E1, beta polypeptide	3E-15	*Rattus norvegicus*	NCBI nr
DBC225	485	1	Sorting nexin associated golgi protein 1	6E-10	*Xenopus laevis*	NCBI nr
DBC233	364	1	Phosphatidylethanolamine binding protein isoform 1	7E-12	*Bombyx mori*	NCBI nr
DBC241	288	1	Vitellogenin	9E-09	*Dermacentor variabilis*	NCBI nr
DBC292	745	1	Glycoprotein 93 similar to CG5520-PA	1E-76	*Nasonia vitripennis*	NCBI nr
DBC326	300	1	ATP/ADP translocase	3E-16	*Marsupenaeus japonicus*	NCBI nr
DBC327	173	1	FUN14 family protein	2E-07	*Ixodes scapularis*	NCBI nr
DBC006	416	13	Vitellogenin_N			InterProScan
DBC011	177	1	Ribosomal protein S14			InterProScan
DBC042	514	3	Immunoglobulin/major histocompatibility complex, conserved site			InterProScan
DBC056	398	1	Zinc finger C2H2-type			InterProScan
DBC060	398	1	Zinc finger C2H2-type			InterProScan
DBC061	671	10	Vitellogenin_N,			InterProScan
DBC093	260	1	Vitellogenin_N,			InterProScan
DBC114	620	1	PROTEIN DISULFIDE ISOMERASE			InterProScan
DBC126	240	1	GTP cyclohydrolase I feedback regulatory protein			InterProScan
DBC149	232	2	Short-chain dehydrogenase/reductase SDR,			InterProScan
DBC154	248	1	Nucleotidyltransferase,			InterProScan
DBC167	325	2	Gonadotropin, beta chain			InterProScan
DBC202	547	1	EGF-like region conserved site			InterProScan

Contig	Length	Nos. of EST	Contig identity/Putative function	*P* value	Species	Database
DBC211	566	1	Protein translocase SEC61 complex gamma subunit,			InterProScan
DBC232	283	1	Aldehyde dehydrogenase conserved site			InterProScan
DBC268	448	1	Zinc finger FYVE/PHD-type			InterProScan
DBC279	313	1	Cysteine proteinases			InterProScan
DBC291	271	1	Scramblase, PHOSPHOLIPID SCRAMBLASE 1, 2			InterProScan
DBC299	770	1	EGF-like region conserved site,			InterProScan
DBC330	769	1	FYVE/PHD zinc finger			InterProScan

Appendix 2:

Roche 454 database split into COG classifications

COG major and sub classifications

INFORMATION STORAGE AND PROCESSING (I)

J Translation, ribosomal structure and biogenesis
A RNA processing and modification
K Transcription
L Replication, recombination and repair
B Chromatin structure and dynamics

CELLULAR PROCESSES AND SIGNALING (C)

D Cell cycle control, cell division, chromosome partitioning
Y Nuclear structure
V Defense mechanisms
T Signal transduction mechanisms
M Cell wall/membrane/envelope biogenesis
N Cell motility
Z Cytoskeleton
W Extracellular structures
U Intracellular trafficking, secretion, and vesicular transport
O Posttranslational modification, protein turnover, chaperones

METABOLISM (M)

C Energy production and conversion
G Carbohydrate transport and metabolism
E Amino acid transport and metabolism
F Nucleotide transport and metabolism
H Coenzyme transport and metabolism
I Lipid transport and metabolism
P Inorganic ion transport and metabolism
Q Secondary metabolites biosynthesis, transport and catabolism

Cellular Processes and Signaling

Contig	BLAST result	e value	Class
contig11065	gi|62425774|ref|ZP_00380905.1| COG0642: Signal transduction hist... 33	9.6	M
contig12124	gi|156388121|ref|XP_001634550.1| predicted protein [*Nematostella*... 33	9.6	U
contig09226	gi|125858048|gb|AAI29157.1| Importin 9 [*Danio rerio*] >gnl|BL_ORD... 33	9.6	UY
contig07751	gi|195393016|ref|XP_002055150.1| GJ19212 [*Drosophila virilis*] >g... 33	9.6	V
contig03882	gi|194385568|dbj|BAG65161.1| unnamed protein product [*Homo sapiens*] 33	9.6	YU
contig08944	gi|123498833|ref|XP_001327486.1| SH3 domain containing protein [... 33	9.5	T
contig06368	gi|159490555|ref|XP_001703240.1| hypothetical protein CHLREDRAFT... 33	9.5	U
contig07940	gi|115751468|ref|XP_785198.2| PREDICTED: similar to laminin B2 [... 33	9.5	W
contig01667	gi|167384655|ref|XP_001737041.1| hypothetical protein, conserved... 33	9.4	O
contig07931	gi|195430620|ref|XP_002063352.1| GK21858 [*Drosophila willistoni*]... 33	9.4	O
contig00791	gi|187039719|emb|CAP21397.1| Hypothetical protein CBG24893 [*Caen*... 33	9.4	T
contig06188	gi|195573070|ref|XP_002104518.1| GD21002 [*Drosophila simulans*] >... 33	9.4	T
contig04878	gi|50546493|ref|XP_500716.1| hypothetical protein [*Yarrowia lipo*... 33	9.4	TR
contig05214	gi|119622371|gb|EAX01966.1| phosphodiesterase 8A, isoform CRA_b ... 33	9.4	TV
contig03416	gi|47218346|emb|CAG04178.1| unnamed protein product [*Tetraodon n*... 33	9.4	W
contig06533	gi|195583169|ref|XP_002081396.1| GD25734 [Drosophila simulans] >... 33	9.4	Z
contig08538	gi|47209492|emb|CAG13281.1| unnamed protein product [*Tetraodon n*... 33	9.3	O
contig04794	gi|72124879|ref|XP_792408.1| PREDICTED: hypothetical protein [*St*... 33	9.3	T
contig07762	gi|52076835|dbj|BAD45777.1| hypothetical protein [*Oryza sativa* J... 33	9.2	U
contig02035	gi|167536190|ref|XP_001749767.1| predicted protein [*Monosiga bre*... 33	8.5	T
contig00341	gi|70951758|ref|XP_745094.1| hypothetical protein PC000597.02.0 ... 33	8	YU
contig01036	gi|157136025|ref|XP_001656733.1| axis inhibition protein, axin [... 33	7.3	O
contig01290	gi|56204188|emb|CAI23045.1| mitogen-activated protein kinase kin... 33	7.3	T
contig11202	gi|167457373|ref|ZP_02323586.1| hypothetical protein A2cp1DRAFT_... 33	7.3	T
contig10533	gi|126290333|ref|XP_001372442.1| PREDICTED: similar to mKIAA0284... 33	7.3	U
contig03404	gi|118091717|ref|XP_001231608.1| PREDICTED: hypothetical protein... 33	7.3	W
contig09639	gi|170049792|ref|XP_001870929.1| conserved hypothetical protein ... 33	7.3	YU
contig01561	gi|33325642|gb|AAQ08323.1| complement component 3-like protein [... 33	7.2	D
contig10900	gi|145478439|ref|XP_001425242.1| hypothetical protein GSPATT0002... 33	7.2	NTZ
contig06835	gi|195377152|ref|XP_002047356.1| GJ11977 [*Drosophila virilis*] >g... 33	7.2	O
contig10182	gi|17560520|ref|NP_505945.1| Temporarily Assigned Gene name fami... 33	7.2	O
contig10375	gi|156546060|ref|XP_001600638.1| PREDICTED: hypothetical protein... 33	7.2	O
contig00698	gi|124512590|ref|XP_001349428.1| hypothetical protein, conserved... 33	7.2	T
contig04761	gi|166998782|ref|ZP_02264636.1| hypothetical protein BmalP_02679... 33	7.2	T
contig01924	gi|115473629|ref|NP_001060413.1| Os07g0639000 [*Oryza sativa* (jap... 33	7.2	V
contig06943	gi|195398411|ref|XP_002057815.1| GJ18339 [Drosophila virilis] >g... 33	7.2	W
contig11746	gi|161526028|ref|YP_001581040.1| phosphoglycolate phosphatase [*B*... 33	7.2	W
contig01056	gi|118099423|ref|XP_415413.2| PREDICTED: similar to quiescin Q6-... 33	7.1	D
contig09411	gi|114555498|ref|XP_001167189.1| PREDICTED: claspin isoform 1 [*P*... 33	7.1	DT
contig04263	gi|157353201|emb|CAO45124.1| unnamed protein product [*Vitis vini*... 33	7.1	MOT
contig11057	gi|195119648|ref|XP_002004342.1| GI19880 [*Drosophila mojavensis*]... 33	7.1	O
contig11121	gi|148675478|gb|EDL07425.1| DnaJ (Hsp40) homolog, subfamily C, m... 33	7.1	O
contig00923	gi|156543188|ref|XP_001606169.1| PREDICTED: similar to slingshot... 33	7.1	T
contig06241	gi|118361871|ref|XP_001014163.1| hypothetical protein TTHERM_002... 33	7.1	T
contig07193	gi|114707159|ref|ZP_01440057.1| hypothetical protein FP2506_0461... 33	7.1	U
contig03953	gi|71980924|ref|NP_001021005.1| Dauer or Aging adult Overexpress... 33	7.1	Y
contig12789	gi|186491181|ref|NP_564696.2| microtubule motor [*Arabidopsis tha*... 33	7.1	Z
contig11123	gi|198133265|gb|EAL29168.2| GA11151 [*Drosophila pseudoobscura* ps... 35	6.5	U
contig12804	gi|126306506|ref|XP_001375368.1| PREDICTED: similar to laminin 5... 34	6.1	W

Contig	BLAST result	e value	Class
contig03923	gi|145254746|ref|XP_001398733.1| hypothetical protein An18g02740... 33	5.6	O
contig07268	gi|182418304|ref|ZP_02949599.1| heat-inducible transcription rep... 33	5.6	O
contig05496	gi|168180139|ref|ZP_02614803.1| hypothetical protein CBN_1627 [*C*... 33	5.6	T
contig06502	gi|118353053|ref|XP_001009797.1| hypothetical protein TTHERM_001... 33	5.6	T
contig09832	gi|194221935|ref|XP_001492983.2| PREDICTED: similar to suppresso... 33	5.6	T
contig11851	gi|167947380|ref|ZP_02534454.1| Fe-S cluster domain protein [*End*... 33	5.6	T
contig12665	gi|120602788|ref|YP_967188.1| methionine aminopeptidase, type I ... 33	5.6	U
contig01680	gi|170070231|ref|XP_001869508.1| conserved hypothetical protein ... 33	5.5	O
contig00864	gi|109480404|ref|XP_001079928.1| PREDICTED: hypothetical protein... 33	5.5	T
contig11588	gi|116060559|emb|CAL55895.1| unnamed protein product [*Ostreococc*... 33	5.5	T
contig04187	gi|50251904|dbj|BAD27842.1| hypothetical protein [*Oryza sativa* J... 33	5.5	Z
contig03434	gi|184155519|ref|YP_001843859.1| methyltransferase [*Lactobacillu*... 33	5.4	D
contig12069	gi|170578730|ref|XP_001894520.1| hypothetical protein Bm1_15300 ... 33	5.4	O
contig12829	gi|171695622|ref|XP_001912735.1| unnamed protein product [*Podosp*... 33	5.4	T
contig00029	gi|177665666|ref|ZP_02941465.1| Tetratricopeptide TPR_2 repeat p... 33	5.4	Z
contig01420	gi|169203868|ref|XP_001714781.1| PREDICTED: hypothetical protein... 34	5.2	O
contig12651	gi|157749721|ref|XP_001678574.1| Hypothetical protein CBG00733 [... 34	4.4	D
contig10527	gi|67153366|ref|ZP_00415111.1| Polysaccharide biosynthesis prote... 34	4.3	NTZ
contig01008	gi|169844679|ref|XP_001829060.1| hypothetical protein CC1G_01740... 34	4.3	O
contig08300	gi|156548302|ref|XP_001602506.1| PREDICTED: similar to protein p... 34	4.3	O
contig10555	gi|198249428|gb|ACH85021.1| ABC-2 type transporter, NodJ family ... 34	4.3	O
contig01382	gi|119113210|ref|XP_309461.3| Toll-like receptor (AGAP011186-PA)... 34	4.3	T
contig09507	gi|125559499|gb|EAZ05035.1| hypothetical protein OsI_026267 [Ory... 34	4.3	T
contig09272	gi|126335686|ref|XP_001370682.1| PREDICTED: hypothetical protein... 34	4.3	U
contig11466	gi|126297966|ref|XP_001371704.1| PREDICTED: hypothetical protein... 34	4.3	U
contig05274	gi|156406751|ref|XP_001641208.1| predicted protein [*Nematostella*... 34	4.3	Z
contig01994	gi|109123769|ref|XP_001112843.1| PREDICTED: hypothetical protein... 34	4.2	D
contig00683	gi|197749971|ref|YP_002173498.1| ABC superfamily ATP binding cas... 34	4.2	MW
contig02065	gi|118581641|ref|YP_902891.1| transglutaminase domain-containing... 34	4.2	O
contig03181	gi|170699244|ref|ZP_02890295.1| hypothetical protein BamIOP4010D... 34	4.2	O
contig10672	gi|25013159|gb|AAN71692.1| SD22693p [*Drosophila melanogaster*] 34	4.2	O
contig11262	gi|91093683|ref|XP_970017.1| PREDICTED: similar to AGAP007113-PA... 34	4.2	O
contig05761	gi|195590932|ref|XP_002085198.1| GD14667 [*Drosophila simulans*] >... 34	4.2	OU
contig00062	gi|73993564|ref|XP_534544.2| PREDICTED: similar to protein tyros... 34	4.2	T
contig01618	gi|77919765|ref|YP_357580.1| adenylylating enzyme for glutamine ... 34	4.2	T
contig04530	gi|111225915|ref|YP_716709.1| signal peptidase-like protein [Fra... 34	4.2	T
contig05567	gi|125549301|gb|EAY95123.1| hypothetical protein OsI_016356 [Ory... 34	4.2	T
contig05732	gi|71659287|ref|XP_821367.1| hypothetical protein Tc00.104705351... 34	4.2	T
contig07731	gi|126734799|ref|ZP_01750545.1| branched-chain amino acid ABC tr... 34	4.2	T
contig12612	gi|153838997|ref|ZP_01991664.1| ATPase component of ABC transpor... 34	4.2	T
contig07030	gi|57864805|gb|AAW56980.1| homocitrate synthase [*Cyanothece* sp. ... 34	4.2	TV
contig12342	gi|193643513|ref|XP_001943927.1| PREDICTED: similar to AGAP01087... 34	4.2	TV
contig05860	gi|190405189|gb|EDV08456.1| COPII coat of secretory pathway vesi... 34	4.2	U
contig06965	gi|169597363|ref|XP_001792105.1| hypothetical protein SNOG_01467... 34	4.2	U
contig04105	gi|70944395|ref|XP_742133.1| hypothetical protein PC104452.00.0 ... 34	4.1	T
contig09582	gi|34393988|dbj|BAC84012.1| hypothetical protein [*Oryza sativa* J... 34	3.3	D
contig08032	gi|71001204|ref|XP_755283.1| eukaryotic translation initiation f... 34	3.3	DZ
contig03622	gi|191161587|ref|ZP_03023469.1| hypothetical protein GM21DRAFT_0... 34	3.3	M
contig08889	gi|193678945|ref|XP_001949533.1| PREDICTED: similar to CG10542 C... 34	3.3	O
contig00125	gi|156606|gb|AAA28264.1| SpId 34	3.3	T
contig09240	gi|149066511|gb|EDM16384.1| hypothetical protein LOC500855, isof... 34	3.3	T

Contig	BLAST result	e value	Class
contig06216	gi|145507812|ref|XP_001439861.1| hypothetical protein GSPATT0000... 34	3.3	Z
contig08151	gi|157746539|ref|XP_001668365.1| Hypothetical protein CBG12357 [... 34	3.2	D
contig06479	gi|45198890|ref|NP_985919.1| AFR372Wp [*Ashbya gossypii* ATCC 1089... 34	3.2	DZ
contig04538	gi|195398873|ref|XP_002058045.1| GJ15706 [*Drosophila virilis*] >g... 34	3.2	O
contig02639	gi|124262623|ref|YP_001023093.1| hypothetical protein Mpe_B0079 ... 34	3.2	T
contig04570	gi|118375060|ref|XP_001020716.1| hypothetical protein TTHERM_012... 34	3.2	T
contig11265	gi|67924417|ref|ZP_00517844.1| Hydrogenase maturation protein Hy... 34	3.2	T
contig02220	gi|167624360|ref|YP_001674654.1| hypothetical protein Shal_2437 ... 34	3.2	TR
contig05803	gi|170070484|ref|XP_001869596.1| phosphatidylinositol 4-kinase [... 34	3.2	TU
contig03821	gi|6320452|ref|NP_010532.1| One of 10 subunits of the transport ... 34	3.2	U
contig11166	gi|195111456|ref|XP_002000294.1| GI10149 [*Drosophila mojavensis*]... 36	3.2	U
contig04131	gi|195034401|ref|XP_001988888.1| GH11411 [*Drosophila grimshawi*] ... 34	3.2	W
contig11258	gi|156398596|ref|XP_001638274.1| predicted protein [*Nematostella*... 34	3.2	W
contig08501	gi|195174473|ref|XP_002027998.1| GL16648 [*Drosophila persimilis*]... 34	3.2	YU
contig04516	gi|118360810|ref|XP_001013636.1| hypothetical protein TTHERM_008... 34	3.2	Z
contig06995	gi|147919261|ref|YP_687003.1| Holliday junction DNA helicase B [... 34	3.2	Z
contig08805	gi|57241961|ref|ZP_00369901.1| conserved hypothetical protein TI... 34	3.2	Z
contig11596	gi|439289|emb|CAA81388.1| verprolin [*Saccharomyces cerevisiae*] 37	3.2	Z
contig05015	gi|90080692|dbj|BAE89827.1| unnamed protein product [*Macaca fasc*... 34	3.2	ZU
contig08129	gi|198150934|gb|EAL30012.2| GA20934 [*Drosophila pseudoobscura* ps... 35	3.1	T
contig01201	gi|157736697|ref|YP_001489380.1| hypothetical protein Abu_0436 [... 35	3.1	W
contig08723	gi|58267468|ref|XP_570890.1| eukaryotic translation initiation f... 35	2.5	D
contig09025	gi|68468367|ref|XP_721743.1| hypothetical protein CaO19.1666 [Ca... 35	2.5	D
contig09784	gi|53792183|dbj|BAD52816.1| vegetative cell wall protein gp1 -li... 35	2.5	D
contig00608	gi|170095453|ref|XP_001878947.1| predicted protein [Laccaria bic... 35	2.5	DO
contig05039	gi|85691079|ref|XP_965939.1| hypothetical protein ECU01_1030 [En... 35	2.5	DZ
contig07600	gi|149242348|ref|XP_001526452.1| hypothetical protein LELG_03010... 35	2.5	M
contig00477	gi|156402682|ref|XP_001639719.1| predicted protein [*Nematostella*... 35	2.5	O
contig10935	gi|67538958|ref|XP_663253.1| hypothetical protein AN5649.2 [*Aspe*... 35	2.5	O
contig12429	gi|114052162|ref|NP_001040460.1| proteasome beta 3 subunit [*Bomb*... 35	2.5	O
contig00827	gi|195121432|ref|XP_002005224.1| GI20375 [*Drosophila mojavensis*]... 37	2.5	T
contig03803	gi|196185300|gb|EDX80276.1| Pyridine nucleotide-disulphide oxido... 35	2.5	T
contig09595	gi|58265682|ref|XP_569997.1| DNA supercoiling [*Cryptococcus neof*... 35	2.5	T
contig09599	gi|170117594|ref|XP_001889983.1| predicted protein [*Laccaria bic*... 35	2.5	T
contig10388	gi|145528738|ref|XP_001450163.1| hypothetical protein GSPATT0001... 35	2.5	T
contig11059	gi|195432721|ref|XP_002064365.1| GK19733 [*Drosophila willistoni*]... 35	2.5	T
contig11341	gi|118082345|ref|XP_416047.2| PREDICTED: similar to SRrp129 [Gal... 35	2.5	T
contig07512	gi|149999616|ref|NP_073293.2| protein LORF2 [*Meleagrid herpesvir*... 35	2.5	TR
contig01591	gi|17569677|ref|NP_509052.1| T07H6.4 [*Caenorhabditis elegans*] >g... 35	2.5	TV
contig07496	gi|147768721|emb|CAN62675.1| hypothetical protein [*Vitis vinifera*] 35	2.5	TZR
contig04020	gi|70935360|ref|XP_738775.1| hypothetical protein PC401526.00.0 ... 35	2.5	W
contig12458	gi|83749245|ref|ZP_00946245.1| Carbon monoxide oxygenase (cytoch... 35	2.5	W
contig06431	gi|145522165|ref|XP_001446932.1| hypothetical protein GSPATT0001... 35	2.5	Z
contig09292	gi|170059945|ref|XP_001865583.1| something about silencing prote... 35	2.5	Z
contig07542	gi|6323273|ref|NP_013345.1| Methionine aminopeptidase, catalyzes... 35	2.4	O
contig01144	gi|156842348|ref|XP_001644542.1| hypothetical protein Kpol_1052p... 35	2.4	V
contig12682	gi|169218160|ref|XP_001719783.1| PREDICTED: similar to KLHL23 pr... 27	2.2	TR
contig00416	gi|67603978|ref|XP_666583.1| hypothetical protein Chro.70593 [Cr... 39	2.1	U
contig01278	gi|195434521|ref|XP_002065251.1| GK15350 [*Drosophila willistoni*]... 37	2	O
contig03680	gi|72387830|ref|XP_844339.1| hypothetical protein, conserved [*Tr*... 35	1.9	D
contig10480	gi|87125211|ref|ZP_01081057.1| putative beta-glucosidase [*Synech*... 35	1.9	DO

Contig	BLAST result	e value	Class
contig00259	gi|161507977|ref|YP_001577941.1| putative ATP-dependent helicase... 35	1.9	O
contig01558	gi|110769117|ref|XP_394836.3| PREDICTED: similar to ubiquitin sp... 35	1.9	O
contig04787	gi|194759065|ref|XP_001961770.1| GF14762 [*Drosophila ananassae*] ... 35	1.9	O
contig07232	gi|147900295|ref|NP_001083439.1| hypothetical protein LOC398926 ... 35	1.9	O
contig08465	gi|121593982|ref|YP_985878.1| hypothetical protein Ajs_1609 [*Aci*... 35	1.9	O
contig10701	gi|156550335|ref|XP_001603253.1| PREDICTED: similar to 26S prote... 35	1.9	O
contig12910	gi|156399835|ref|XP_001638706.1| predicted protein [*Nematostella*... 35	1.9	O
contig13073	gi|156392707|ref|XP_001636189.1| predicted protein [*Nematostella*... 35	1.9	O
contig02661	gi|188026387|ref|ZP_02997884.1| hypothetical protein PROSTU_0397... 35	1.9	T
contig04865	gi|167525154|ref|XP_001746912.1| predicted protein [*Monosiga bre*... 30	1.9	T
contig07719	gi|194895594|ref|XP_001978292.1| GG17765 [*Drosophila erecta*] >gn... 35	1.9	T
contig00598	gi|190574310|ref|YP_001972155.1| putative two-component response... 35	1.9	TZ
contig03948	gi|195388260|ref|XP_002052801.1| GJ19836 [*Drosophila virilis*] >g... 35	1.9	U
contig01264	gi|194766886|ref|XP_001965555.1| GF22554 [*Drosophila ananassae*] ... 35	1.5	D
contig05034	gi|46110719|ref|XP_382417.1| hypothetical protein FG02241.1 [Gib... 35	1.5	O
contig07484	gi|194367365|ref|YP_002029975.1| hypothetical protein Smal_3593 ... 35	1.5	O
contig08622	gi|148680449|gb|EDL12396.1| mCG125898 [*Mus musculus*] 35	1.5	O
contig09236	gi|193787650|dbj|BAG52856.1| unnamed protein product [*Homo sapiens*] 35	1.5	O
contig11714	gi|67522775|ref|XP_659448.1| hypothetical protein AN1844.2 [*Aspe*... 35	1.5	O
contig01340	gi|77022988|ref|XP_888938.1| hypothetical protein CaO19_1346 [Ca... 37	1.5	OT
contig00676	gi|195576554|ref|XP_002078140.1| GD22701 [*Drosophila simulans*] >... 35	1.5	T
contig07290	gi|50550919|ref|XP_502933.1| hypothetical protein [*Yarrowia lipo*... 35	1.5	T
contig11279	gi|67901420|ref|XP_680966.1| hypothetical protein AN7697.2 [*Aspe*... 35	1.5	T
contig04177	gi|89897355|ref|YP_520842.1| putative oxidoreductase iron-sulfur... 35	1.5	TU
contig11852	gi|170699604|ref|ZP_02890643.1| conserved hypothetical protein [... 35	1.5	W
contig02884	gi|13487281|gb|AAK27473.1| polyketide synthase; SvKS [*Streptomyc*... 35	1.5	Z
contig02569	gi|194697734|gb|ACF82951.1| unknown [*Zea mays*] 37	1.4	MW
contig13298	gi|115455821|ref|NP_001051511.1| Os03g0789600 [*Oryza sativa* (jap... 38	1.4	N
contig06288	gi|195131669|ref|XP_002010268.1| GI14785 [*Drosophila mojavensis*]... 35	1.4	T
contig11172	gi|76162433|gb|AAX30253.2| SJCHGC02429 protein [*Schistosoma japo*... 30	1.4	T
contig05814	gi|73988191|ref|XP_542325.2| PREDICTED: similar to centaurin del... 35	1.4	TU
contig03391	gi|19718366|ref|NP_604435.1| phosphoprotein [Human parainfluenza... 35	1.4	TZ
contig01838	gi|204787|gb|AAA41396.1| Immunoglobulin kappa-chain VJ precursor 35	1.4	U
contig09717	gi|167386146|ref|XP_001737636.1| hypothetical protein, conserved... 35	1.4	U
contig04048	gi|156554278|ref|XP_001602116.1| PREDICTED: similar to ENSANGP00... 35	1.4	W
contig04916	gi|126002135|ref|XP_001352272.1| GA14535-PA [*Drosophila pseudoob*... 28	1.3	M
contig03638	gi|125591112|gb|EAZ31462.1| hypothetical protein OsJ_014945 [Ory... 38	1.3	W
contig03608	gi|195344163|ref|XP_002038658.1| GM10939 [*Drosophila sechellia*] ... 37	1.3	Z
contig05694	gi|195574457|ref|XP_002105205.1| GD21359 [*Drosophila simulans*] >... 36	1.1	O
contig12078	gi|158289547|ref|XP_311247.4| AGAP000713-PA [*Anopheles gambiae* s... 36	1.1	O
contig01095	gi|162454494|ref|YP_001616861.1| hypothetical protein sce6214 [*S*... 36	1.1	T
contig02428	gi|116492183|ref|YP_803918.1| x-prolyl-dipeptidyl aminopeptidase... 36	1.1	T
contig06549	gi|164661015|ref|XP_001731630.1| hypothetical protein MGL_0898 [... 36	1.1	T
contig03290	gi|156546770|ref|XP_001605588.1| PREDICTED: similar to rap55 [*Na*... 36	1.1	U
contig06597	gi|47216957|emb|CAG04899.1| unnamed protein product [*Tetraodon n*... 36	1.1	U
contig08524	gi|159481730|ref|XP_001698931.1| hypothetical protein CHLREDRAFT... 36	1.1	U
contig03324	gi|193702277|ref|XP_001948755.1| PREDICTED: similar to AGAP00204... 36	1.1	V
contig10773	gi|19115005|ref|NP_594093.1| nucleoporin Nup189 [*Schizosaccharom*... 36	1.1	YU
contig05865	gi|195161123|ref|XP_002021419.1| GL25318 [*Drosophila persimilis*]... 36	1.1	Z
contig08209	gi|70798578|gb|AAZ08999.1| immunoglobulin heavy chain variable r... 36	1.1	Z
contig02054	gi|39957692|ref|XP_364348.1| hypothetical protein MGG_09193 [Mag... 36	0.97	N

Contig	BLAST result	e value	Class
contig03270	gi|116793592|gb|ABK26801.1| unknown [*Picea sitchensis*] 37	0.9	T
contig08553	gi|156383791|ref|XP_001633016.1| predicted protein [*Nematostella*... 36	0.87	O
contig02900	gi|85090533|ref|XP_958462.1| hypothetical protein NCU09955 [*Neur*... 36	0.86	O
contig01757	gi|114615998|ref|XP_001135989.1| PREDICTED: plexin A4, A isoform... 36	0.86	T
contig06286	gi|169640128|gb|ACA61137.1| cellulase [uncultured microorganism] 36	0.86	T
contig10563	gi|73965496|ref|XP_548054.2| PREDICTED: similar to C1q-related f... 36	0.86	T
contig12323	gi|124504889|ref|XP_001351187.1| putative protein kinase [*Plasmo*... 36	0.86	W
contig10528	gi|157676123|emb|CAP08065.1| m21 protein [Murine cytomegalovirus... 36	0.85	O
contig03058	gi|197925298|ref|ZP_03190244.1| hypothetical protein SpriA_20024... 36	0.85	T
contig05959	gi|193681063|ref|XP_001950378.1| PREDICTED: similar to chromaffi... 36	0.85	U
contig08521	gi|156366295|ref|XP_001627074.1| predicted protein [*Nematostella*... 36	0.85	U
contig10018	gi|152989514|ref|YP_001346511.1| hypothetical protein PSPA7_1125... 36	0.85	W
contig00739	gi|116198151|ref|XP_001224887.1| hypothetical protein CHGG_07231... 36	0.85	Z
contig04127	gi|170575044|ref|XP_001893074.1| Twik (KCNK-like) family of pota... 36	0.84	M
contig00264	gi|156359643|ref|XP_001624876.1| predicted protein [*Nematostella*... 36	0.84	O
contig00075	gi|42761547|gb|AAS45365.1| similar to *Plasmodium falciparum*. Hyp... 38	0.77	Z
contig12607	gi|157865433|ref|XP_001681424.1| hypothetical protein, conserved... 39	0.73	TZ
contig05086	gi|168031786|ref|XP_001768401.1| predicted protein [*Physcomitrel*... 28	0.7	O
contig07878	gi|145253362|ref|XP_001398194.1| hypothetical protein An16g08960... 37	0.66	D
contig13323	gi|119719782|ref|YP_920277.1| hypothetical protein Tpen_0873 [*Th*... 37	0.66	O
contig00491	gi|195386888|ref|XP_002052136.1| GJ17388 [*Drosophila virilis*] >g... 37	0.66	T
contig01028	gi|114625625|ref|XP_001151769.1| PREDICTED: similar to basic pro... 37	0.66	T
contig10520	gi|170585792|ref|XP_001897666.1| Protein kinase c protein 2, iso... 37	0.66	T
contig12170	gi|182680179|ref|YP_001834325.1| hypothetical protein Bind_3276 ... 37	0.65	O
contig13052	gi|73955932|ref|XP_863640.1| PREDICTED: similar to ubiquitin B p... 37	0.65	OR
contig04523	gi|148706314|gb|EDL38261.1| EGF-like module containing, mucin-li... 37	0.65	T
contig11739	gi|153833093|ref|ZP_01985760.1| hypothetical protein A1Q_1062 [V... 37	0.65	T
contig05894	gi|115379274|ref|ZP_01466387.1| glutamine synthetase [*Stigmatell*... 37	0.65	Z
contig01483	gi|66814134|ref|XP_641246.1| hypothetical protein DDBDRAFT_02182... 37	0.64	D
contig01088	gi|198139346|gb|EAL33262.2| GA13628 [*Drosophila pseudoobscura* ps... 37	0.64	O
contig08922	gi|169860246|ref|XP_001836758.1| hypothetical protein CC1G_04071... 37	0.64	OU
contig01623	gi|167357703|ref|ZP_02292367.1| methyltransferase-like protein [... 37	0.64	U
contig07023	gi|157125996|ref|XP_001654485.1| hypothetical protein AaeL_AAEL0... 31	0.62	T
contig00475	gi|18027726|gb|AAL55824.1|AF318317_1 unknown [*Homo sapiens*] 28	0.6	T
contig12885	gi|149919653|ref|ZP_01908132.1| hypothetical protein PPSIR1_0726... 38	0.52	T
contig03576	gi|157344388|emb|CAO69771.1| unnamed protein product [*Vitis vini*... 37	0.51	D
contig08022	gi|169170317|ref|XP_001718125.1| PREDICTED: hypothetical protein... 28	0.51	T
contig08382	gi|189534684|ref|XP_001919656.1| PREDICTED: similar to zinc fing... 37	0.51	T
contig12728	gi|117957263|gb|ABK59062.1| polymerase [Human adenovirus 37] 37	0.5	O
contig01782	gi|119873111|ref|YP_931118.1| saccharopine dehydrogenase [*Pyroba*... 37	0.5	T
contig07215	gi|115894447|ref|XP_780669.2| PREDICTED: similar to DBC2 protein... 37	0.5	TZ
contig04534	gi|110761227|ref|XP_001122167.1| PREDICTED: similar to CG4030-PA... 37	0.5	U
contig05499	gi|147845679|emb|CAN80589.1| hypothetical protein [*Vitis vinifera*] 37	0.5	U
contig06902	gi|194757768|ref|XP_001961134.1| GF11157 [*Drosophila ananassae*] ... 37	0.5	U
contig00284	gi|45184624|gb|AAS55564.1| MST84DC [*Drosophila mauritiana*] >gnl|... 37	0.5	W
contig03233	gi|12249142|ref|NP_065432.2| NADH dehydrogenase subunit 6 [*Schis*... 37	0.49	T
contig01823	gi|1335202|emb|CAA56130.1| beta2/S laminin chain [*Homo sapiens*] 37	0.46	W
contig08825	gi|157136051|ref|XP_001656746.1| signal recognition particle [*Ae*... 37	0.39	U
contig11680	gi|123504770|ref|XP_001328828.1| LIM domain containing protein [... 37	0.39	W
contig09447	gi|156548864|ref|XP_001606345.1| PREDICTED: similar to MGC80357 ... 37	0.38	T
contig10615	gi|148681709|gb|EDL13656.1| mCG15239 [*Mus musculus*] 37	0.38	T

Contig	BLAST result	e value	Class
contig11661	gi|71749482|ref|XP_828080.1| hypothetical protein Tb10.61.0160 [... 37	0.38	Z
contig12765	gi|125591402|gb|EAZ31752.1| hypothetical protein OsJ_015235 [Ory... 40	0.31	D
contig01255	gi|189237968|ref|XP_001811946.1| PREDICTED: similar to CG8155 CG... 38	0.3	T
contig02256	gi|189202442|ref|XP_001937557.1| conserved hypothetical protein ... 38	0.3	U
contig07132	gi|195491533|ref|XP_002093601.1| ida [*Drosophila yakuba*] >gnl|BL... 38	0.29	DO
contig06957	gi|29841035|gb|AAP06048.1| similar to NM_017284 proteasome (pros... 38	0.29	O
contig03938	gi|169173427|ref|XP_001717734.1| PREDICTED: hypothetical protein... 38	0.29	T
contig09368	gi|157123175|ref|XP_001660044.1| cd36 antigen [*Aedes aegypti*] >g... 38	0.29	T
contig02786	gi|189523564|ref|XP_001920789.1| PREDICTED: hypothetical protein... 38	0.29	TV
contig01211	gi|125586705|gb|EAZ27369.1| hypothetical protein OsJ_010852 [Ory... 40	0.26	TV
contig04335	gi|194218680|ref|XP_001494740.2| PREDICTED: similar to Actin-rel... 38	0.24	Z
contig10538	gi|167523042|ref|XP_001745858.1| predicted protein [Monosiga bre... 38	0.23	DZU
contig00231	gi|156550801|ref|XP_001600558.1| PREDICTED: similar to FTP3 [Nas... 38	0.23	O
contig05696	gi|168067485|ref|XP_001785646.1| predicted protein [*Physcomitrel*... 38	0.23	O
contig09541	gi|170033288|ref|XP_001844510.1| olygosaccharyltransferase alpha... 38	0.23	O
contig00118	gi|125531144|gb|EAY77709.1| hypothetical protein OsI_031668 [*Ory*... 38	0.23	T
contig02828	gi|194761414|ref|XP_001962924.1| GF15679 [*Drosophila ananassae*] ... 39	0.23	T
contig05395	gi|149616040|ref|XP_001510974.1| PREDICTED: similar to hCG204480... 39	0.23	T
contig05780	gi|161661029|gb|ABX75381.1| cysteine and glycine-rich protein [L... 38	0.23	TZ
contig03739	gi|148668868|gb|EDL01036.1| mCG129264 [*Mus musculus*] 38	0.22	DZU
contig03206	gi|156387705|ref|XP_001634343.1| predicted protein [*Nematostella*... 38	0.22	O
contig05189	gi|114612501|ref|XP_518982.2| PREDICTED: basic leucine zipper an... 38	0.22	O
contig03327	gi|154757410|gb|AAI51785.1| DIAPH3 protein [*Bos taurus*] 38	0.22	T
contig07147	gi|195480495|ref|XP_002101281.1| GE17532 [*Drosophila yakuba*] >gn... 38	0.22	T
contig12673	gi|195168069|ref|XP_002024854.1| GL17883 [*Drosophila persimilis*]... 40	0.22	T
contig09674	gi|156385188|ref|XP_001633513.1| predicted protein [*Nematostella*... 38	0.22	U
contig01224	gi|195029723|ref|XP_001987721.1| GH19811 [*Drosophila grimshawi*] ... 38	0.22	Z
contig11426	gi|198145779|gb|EAL32566.2| GA22026 [*Drosophila pseudoobscura* ps... 40	0.21	T
contig11599	gi|157117680|ref|XP_001658884.1| polybromo-1 [*Aedes aegypti*] >gn... 39	0.19	O
contig01915	gi|4886294|emb|CAB43342.1| Lamin [*Astropecten brasiliensis*] 39	0.17	DY
contig08072	gi|195152453|ref|XP_002017151.1| GL22150 [*Drosophila persimilis*]... 39	0.17	O
contig09138	gi|187608119|ref|NP_001120050.1| hypothetical protein LOC1001450... 39	0.17	O
contig10875	gi|126323246|ref|XP_001376008.1| PREDICTED: similar to scaffold ... 39	0.17	O
contig10154	gi|183987808|gb|ACC65888.1| Down syndrome cell adhesion molecule... 39	0.17	T
contig11014	gi|67845963|dbj|BAE00009.1| LDL receptor [*Seriola quinqueradiata*] 39	0.17	T
contig11182	gi|147901940|ref|NP_001090248.1| hypothetical protein LOC779153 ... 39	0.17	T
contig07097	gi|116871854|ref|YP_848635.1| hypothetical protein lwe0434 [List... 39	0.17	Y
contig07550	gi|118378274|ref|XP_001022313.1| Kinesin motor domain containing... 39	0.17	Z
contig07635	gi|115958669|ref|XP_001194281.1| PREDICTED: hypothetical protein... 39	0.17	Z
contig13245	gi|30023940|ref|NP_835282.1| polymerase [Indian peanut clump virus] 39	0.16	T
contig06692	gi|46559756|ref|NP_956213.2| adaptor-related protein complex 2, ... 32	0.15	U
contig00333	gi|54400522|ref|NP_001006010.1| hypothetical protein LOC449989 [... 39	0.13	DV
contig06046	gi|68063199|ref|XP_673609.1| hypothetical protein PB405521.00.0 ... 39	0.13	M
contig05828	gi|91092020|ref|XP_970897.1| PREDICTED: similar to putative cath... 39	0.13	O
contig07211	gi|93003312|tpd|FAA00239.1| TPA: zinc finger protein [*Ciona inte*... 39	0.13	O
contig08440	gi|158292142|ref|XP_001237526.2| AGAP004413-PA [*Anopheles gambia*... 39	0.13	O
contig04678	gi|114685214|ref|XP_001157109.1| PREDICTED: hypothetical protein... 39	0.13	T
contig00929	gi|195453690|ref|XP_002073897.1| GK14360 [*Drosophila willistoni*]... 39	0.13	U
contig13254	gi|151337348|gb|ABS01106.1| D-hordein [*Hordeum chilense*] 40	0.13	V
contig07893	gi|126338312|ref|XP_001373693.1| PREDICTED: hypothetical protein... 39	0.13	W
contig00182	gi|170112414|ref|XP_001887409.1| predicted protein [*Laccaria bic*... 39	0.13	WV

Contig	BLAST result	e value	Class
contig01861	gi|47221621|emb|CAF97886.1| unnamed protein product [*Tetraodon n*... 39	0.13	Z
contig05576	gi|170581235|ref|XP_001895597.1| tubulin alpha-2 chain [*Brugia m*... 39	0.13	Z
contig03647	gi|5052414|gb|AAD38537.1|AF145029_1 transportin-SR [*Homo sapiens*] 35	0.12	U
contig06590	gi|118785594|ref|XP_314763.3| AGAP008664-PA [*Anopheles gambiae* s... 39	0.1	M
contig03512	gi|156546773|ref|XP_001607297.1| PREDICTED: similar to ubiquitin... 39	0.1	O
contig05102	gi|195483891|ref|XP_002090473.1| GE12790 [*Drosophila yakuba*] >gn... 39	0.1	T
contig11339	gi|116669193|ref|YP_830126.1| nuclear export factor GLE1 [Arthro... 39	0.1	T
contig07353	gi|159472306|ref|XP_001694292.1| predicted protein [*Chlamydomona*... 39	0.1	TR
contig02973	gi|194206111|ref|XP_001491687.2| PREDICTED: similar to Mitochond... 39	0.1	U
contig12070	gi|193643549|ref|XP_001948352.1| PREDICTED: similar to rab6 isof... 39	0.1	U
contig05482	gi|167525597|ref|XP_001747133.1| predicted protein [*Monosiga bre*... 39	0.1	W
contig07698	gi|91091176|ref|XP_971655.1| PREDICTED: similar to S1 RNA bindin... 39	0.1	WV
contig08555	gi|47205490|emb|CAF90253.1| unnamed protein product [*Tetraodon n*... 39	0.10	DZU
contig08637	gi|189233774|ref|XP_001814482.1| PREDICTED: similar to MGC80357 ... 39	0.10	T
contig03121	gi|115770339|ref|XP_001176616.1| PREDICTED: similar to egg bindi... 40	0.08	T
contig10036	gi|198138857|gb|EAL34132.2| GA19606 [*Drosophila pseudoobscura* ps... 40	0.08	T
contig09100	gi|21593914|gb|AAM65879.1| unknown [*Arabidopsis thaliana*] 40	0.08	MOU
contig03378	gi|123477829|ref|XP_001322080.1| hypothetical protein TVAG_25938... 40	0.08	T
contig12024	gi|156451854|ref|ZP_02058228.1| formate dehydrogenase, alpha sub... 40	0.08	O
contig10609	gi|133777115|gb|AAH91093.2| GRN protein [*Xenopus tropicalis*] 40	0.08	T
contig04859	gi|118101636|ref|XP_417771.2| PREDICTED: similar to Arginine/pro... 40	0.06	Z
contig10399	gi|170034430|ref|XP_001845077.1| 26S proteasome non-ATPase regul... 40	0.06	O
contig08563	gi|91095293|ref|XP_967766.1| PREDICTED: similar to calcyclin bin... 40	0.06	T
contig04026	gi|67469205|ref|XP_650594.1| RNA methyltransferase, putative [*En*... 40	0.06	T
contig05609	gi|115371999|ref|ZP_01459311.1| FHA domain protein [*Stigmatella* ... 41	0.06	W
contig08195	gi|189530984|ref|XP_001920524.1| PREDICTED: intersectin like [*Da*... 40	0.05	O
contig03194	gi|119912012|ref|XP_001256523.1| PREDICTED: similar to tetratric... 40	0.05	Z
contig09035	gi|169768980|ref|XP_001818960.1| hypothetical protein [*Aspergill*... 40	0.05	T
contig07655	gi|91092816|ref|XP_967541.1| PREDICTED: similar to AGAP002470-PA... 40	0.05	W
contig06135	gi|153812578|ref|ZP_01965246.1| hypothetical protein RUMOBE_0297... 42	0.04	T
contig12862	gi|194753720|ref|XP_001959158.1| GF12744 [*Drosophila ananassae*] ... 41	0.04	YU
contig11360	gi|33112616|gb|AAF37724.3|AF238234_1 diaphanous protein [*Entamoe*... 42	0.04	W
contig02805	gi|195135429|ref|XP_002012135.1| GI16804 [*Drosophila mojavensis*]... 41	0.04	O
contig01236	gi|123989726|gb|ABM83891.1| mitogen-activated protein-binding pr... 41	0.04	T
contig02975	gi|149264699|ref|XP_001475752.1| PREDICTED: hypothetical protein... 41	0.04	T
contig05292	gi|196002321|ref|XP_002111028.1| hypothetical protein TRIADDRAFT... 41	0.04	U
contig01728	gi|170044717|ref|XP_001849983.1| puromycin-sensitive aminopeptid... 41	0.04	Z
contig05843	gi|118085859|ref|XP_001235427.1| PREDICTED: hypothetical protein... 41	0.04	Z
contig11827	gi|160213448|gb|ABX10976.1| Rho GTPase activating protein 4 (pre... 41	0.04	Z
contig02733	gi|30681147|ref|NP_172416.2| haspin-related [Arabidopsis thalian... 41	0.03	D
contig08295	gi|168013032|ref|XP_001759205.1| predicted protein [*Physcomitrel*... 41	0.03	T
contig09596	gi|156552361|ref|XP_001602164.1| PREDICTED: similar to IP17403p ... 41	0.03	T
contig09310	gi|91081667|ref|XP_969700.1| PREDICTED: similar to AGAP000550-PA... 41	0.03	W
contig02951	gi|149287066|gb|ABR23432.1| alpha crystallin [*Ornithodoros parkeri*] 42	0.03	O
contig09458	gi|47215439|emb|CAG05726.1| unnamed protein product [*Tetraodon n*... 41	0.03	T
contig01380	gi|197246549|gb|AAI68475.1| Unknown (protein for MGC:172737) [*Xe*... 41	0.03	T
contig09956	gi|193673870|ref|XP_001944375.1| PREDICTED: similar to alpha Cat... 41	0.03	W
contig02558	gi|197128346|gb|ACH44844.1| putative dynein cytoplasmic light pe... 43	0.02	Z
contig07791	gi|62857002|dbj|BAD95886.1| Ca/calmodulin-dependent protein kina... 42	0.02	T
contig11765	gi|196000721|ref|XP_002110228.1| expressed hypothetical protein ... 42	0.02	T
contig06506	gi|194668988|ref|XP_001787651.1| PREDICTED: similar to diaphanou... 42	0.02	TZ

Contig	BLAST result	e value	Class
contig00809	gi|157114115|ref|XP_001652166.1| abc transporter [*Aedes aegypti*]... 42	0.02	U
contig11549	gi|145503637|ref|XP_001437793.1| hypothetical protein GSPATT0003... 43	0.02	W
contig08356	gi|110750886|ref|XP_395543.3| PREDICTED: similar to dalao CG7055... 42	0.02	O
contig10447	gi|159060|gb|AAA29140.1| mu-glutathione transferase [*Fasciola he*... 42	0.02	O
contig12559	gi|195567675|ref|XP_002107384.1| GD17434 [*Drosophila simulans*] >... 44	0.02	T
contig04548	gi|193664496|ref|XP_001943041.1| PREDICTED: similar to short sto... 42	0.02	Z
contig03656	gi|195108637|ref|XP_001998899.1| GI24219 [*Drosophila mojavensis*]... 43	0.02	T
contig05325	gi|195047032|ref|XP_001992258.1| GH24301 [*Drosophila grimshawi*] ... 42	0.02	T
contig09169	gi|76801260|ref|YP_326268.1| chromosome partition protein [*Natro*... 42	0.02	Z
contig10090	gi|152996239|ref|YP_001341074.1| hypothetical protein Mmwyl1_221... 45	0.01	O
contig08827	gi|58332124|ref|NP_001011214.1| cathepsin S [*Xenopus* (*Silurana*) ... 42	0.01	O
contig08880	gi|194668814|ref|XP_590284.4| PREDICTED: similar to MPN domain-c... 36	0.01	O
contig04139	gi|156337939|ref|XP_001619924.1| hypothetical protein NEMVEDRAFT... 42	0.01	T
contig00401	gi|156386140|ref|XP_001633771.1| predicted protein [*Nematostella*... 42	0.01	U
contig07786	gi|91094489|ref|XP_971063.1| PREDICTED: similar to CG2095 CG2095... 42	0.01	U
contig11380	gi|170030690|ref|XP_001843221.1| filamin [*Culex quinquefasciatus*... 42	0.01	Z
contig01078	gi|198145578|gb|EDY72179.1| GA22956 [*Drosophila pseudoobscura* ps... 44	0.01	V
contig01447	gi|156552511|ref|XP_001599980.1| PREDICTED: similar to cathepsin... 43	0.01	O
contig03077	gi|170067598|ref|XP_001868545.1| phosphatase and actin regulator... 43	0.01	O
contig08275	gi|196008375|ref|XP_002114053.1| hypothetical protein TRIADDRAFT... 43	0.01	O
contig08476	gi|156553970|ref|XP_001602910.1| PREDICTED: similar to ENSANGP00... 40	0.01	T
contig08986	gi|14647143|gb|AAK71863.1|AF339157_1 mannose-6-phosphate/insulin... 43	0.01	TU
contig09642	gi|195438034|ref|XP_002066942.1| GK24746 [*Drosophila willistoni*]... 43	0.01	YU
contig01564	gi|91094875|ref|XP_972773.1| PREDICTED: similar to CG7394 CG7394... 43	0.01	O
contig02620	gi|149371336|ref|ZP_01890822.1| oligopeptidase (protease II) [un... 43	0.01	O
contig08690	gi|156365829|ref|XP_001626845.1| predicted protein [*Nematostella*... 43	0.01	O
contig10431	gi|169217053|ref|XP_001714101.1| PREDICTED: similar to ubiquitin... 43	0.01	O
contig05787	gi|195553265|ref|XP_002076633.1| GD15097 [*Drosophila simulans*] >... 43	0.01	T
contig03258	gi|118088968|ref|XP_419917.2| PREDICTED: similar to CUB and Sush... 43	0.01	TV
contig01233	gi|149445072|ref|XP_001518993.1| PREDICTED: hypothetical protein... 43	0.01	U
contig09428	gi|195132985|ref|XP_002010920.1| GI21806 [*Drosophila mojavensis*]... 44	0.01	DO
contig03565	gi|159184548|ref|NP_353970.2| hypothetical protein Atu0950 [*Agro*... 44	0.01	O
contig05837	gi|50295084|ref|XP_449953.1| hypothetical protein CAGL0M14003g [... 44	0.01	O
contig09403	gi|156379923|ref|XP_001631705.1| predicted protein [*Nematostella*... 44	0.01	O
contig06804	gi|149056871|gb|EDM08302.1| protein kinase D2 [*Rattus norvegicus*] 44	0.01	T
contig01162	gi|47217245|emb|CAF96768.1| unnamed protein product [*Tetraodon* n... 44	0.01	U
contig05737	gi|195440772|ref|XP_002068214.1| GK10106 [*Drosophila willistoni*]... 44	0.01	Z
contig02758	gi|72030199|ref|XP_780027.1| PREDICTED: similar to MGC80929 prot... 44	0.00	O
contig04135	gi|67597810|ref|XP_666173.1| hypothetical protein Chro.10099 [Cr... 44	0.00	O
contig09154	gi|156401589|ref|XP_001639373.1| predicted protein [*Nematostella*... 44	0.00	O
contig04486	gi|156551402|ref|XP_001603645.1| PREDICTED: similar to 28S ribos... 44	0.00	D
contig05185	gi|195452950|ref|XP_002073572.1| GK13074 [*Drosophila willistoni*]... 44	0.00	D
contig01066	gi|47227523|emb|CAG04671.1| unnamed protein product [*Tetraodon n*... 44	0.00	T
contig10187	gi|195996897|ref|XP_002108317.1| hypothetical protein TRIADDRAFT... 44	0.00	T
contig02735	gi|195479726|ref|XP_002101004.1| GE17372 [*Drosophila yakuba*] >gn... 44	0.00	U
contig08957	gi|110767589|ref|XP_001121853.1| PREDICTED: similar to Transloca... 44	0.00	U
contig08856	gi|159475749|ref|XP_001695981.1| flagellar associated protein, p... 44	0.00	V
contig05991	gi|126329141|ref|XP_001367459.1| PREDICTED: similar to proteasom... 45	0.00	O
contig06638	gi|115687856|ref|XP_001196828.1| PREDICTED: similar to BRCA1/BRC... 45	0.00	O
contig06688	gi|156551191|ref|XP_001604758.1| PREDICTED: similar to phosphati... 45	0.00	O
contig04379	gi|193647994|ref|XP_001946832.1| PREDICTED: similar to protein k... 45	0.00	T

Contig	BLAST result	e value	Class
contig08063	gi|196000721|ref|XP_002110228.1| expressed hypothetical protein ... 45	0.00	T
contig09751	gi|154335798|ref|XP_001564135.1| hypothetical protein LbrM19_V2.... 45	0.00	T
contig12371	gi|149723703|ref|XP_001489791.1| PREDICTED: granulin [*Equus caba*... 45	0.00	T
contig10604	gi|114431574|gb|ABI74628.1| beta-catenin [*Podocoryne carnea*] 45	0.00	TZ
contig04157	gi|170036955|ref|XP_001846326.1| gamma-soluble nsf attachment pr... 45	0.00	U
contig05450	gi|89258423|gb|ABD65435.1| Rab11 [*Suberites domuncula*] 45	0.00	U
contig08623	gi|91084541|ref|XP_972999.1| PREDICTED: similar to vacuolar prot... 45	0.00	U
contig10812	gi|47229714|emb|CAG06910.1| unnamed protein product [*Tetraodon n*... 45	0.00	Z
contig09115	gi|169641795|gb|AAI60392.1| Unknown (protein for IMAGE:7548775) ... 46	0.00	D
contig10842	gi|170059103|ref|XP_001865214.1| VPRBP protein [*Culex quinquefas*... 45	0.00	D
contig08711	gi|149635870|ref|XP_001513294.1| PREDICTED: similar to asparagin... 45	0.00	M
contig07478	gi|93003048|tpd|FAA00107.1| TPA: zinc finger protein [*Ciona inte*... 46	0.00	O
contig11516	gi|170035373|ref|XP_001845544.1| conserved hypothetical protein ... 46	0.00	T
contig05568	gi|170571409|ref|XP_001891717.1| hypothetical protein Bm1_00995 ... 45	0.00	U
contig07874	gi|91090696|ref|XP_974756.1| PREDICTED: similar to cop-coated ve... 46	0.00	U
contig01910	gi|50540242|ref|NP_001002588.1| SEC63-like protein [*Danio rerio*]... 46	0.00	UO
contig06735	gi|156551334|ref|XP_001602109.1| PREDICTED: similar to ENSANGP00... 46	0.00	Z
contig07678	gi|195338965|ref|XP_002036092.1| GM13447 [*Drosophila sechellia*] ... 35	9E-04	O
contig11351	gi|118101683|ref|XP_417788.2| PREDICTED: similar to CSMD2 protei... 48	9E-04	TV
contig07633	gi|195051586|ref|XP_001993128.1| GH13251 [*Drosophila grimshawi*] ... 46	8E-04	M
contig07570	gi|156546249|ref|XP_001605335.1| PREDICTED: similar to ubiquitin... 46	8E-04	O
contig05687	gi|110763741|ref|XP_394054.3| PREDICTED: similar to MAP kinase k... 46	8E-04	T
contig11281	gi|158299368|ref|XP_319468.3| AGAP010276-PA [*Anopheles gambiae* s... 44	8E-04	T
contig02232	gi|5833948|gb|AAD53751.1|AF172845_1 death-associated LIM only pr... 46	8E-04	TZ
contig07278	gi|91086435|ref|XP_968298.1| PREDICTED: similar to HIF prolyl hy... 47	6E-04	T
contig00391	gi|41055654|ref|NP_956490.1| hypothetical protein LOC393165 isof... 47	6E-04	TZ
contig12145	gi|156254836|gb|ABU62829.1| serpin-2 [*Spodoptera exigua*] 47	6E-04	V
contig08682	gi|156385392|ref|XP_001633614.1| predicted protein [*Nematostella*... 47	5E-04	D
contig05867	gi|170064305|ref|XP_001867470.1| cathepsin l [*Culex quinquefasci*... 47	5E-04	O
contig07982	gi|170047354|ref|XP_001851189.1| conserved hypothetical protein ... 47	5E-04	O
contig09608	gi|195119099|ref|XP_002004069.1| GI18251 [*Drosophila mojavensis*]... 47	5E-04	O
contig02208	gi|17555058|ref|NP_499811.1| T12D8.8 [*Caenorhabditis elegans*] >g... 47	5E-04	OT
contig10805	gi|21539615|ref|NP_081110.1| hypothetical protein LOC68768 [*Mus* ... 47	5E-04	W
contig05438	gi|157105151|ref|XP_001648739.1| kinesin-like protein Klp10A [Ae... 47	5E-04	Z
contig09078	gi|195502445|ref|XP_002098227.1| GE24065 [*Drosophila yakuba*] >gn... 47	4E-04	M
contig01198	gi|45386075|gb|AAS59855.1| KOG1656-like protein [*Ornithodoros mo*... 47	4E-04	U
contig02293	gi|157120931|ref|XP_001659800.1| slit protein [*Aedes aegypti*] >g... 47	4E-04	WT
contig09920	gi|198138224|gb|EDY70016.1| GA25794 [*Drosophila pseudoobscura* ps... 34	4E-04	Z
contig02685	gi|72027916|ref|XP_779916.1| PREDICTED: similar to MGC81823 prot... 48	3E-04	O
contig00512	gi|156547498|ref|XP_001605935.1| PREDICTED: similar to ENSANGP00... 48	3E-04	OT
contig05631	gi|170047098|ref|XP_001851073.1| serine/threonine protein kinase... 48	3E-04	T
contig03542	gi|27369487|ref|NP_765985.1| LIM domain containing 2 [*Mus muscul*... 48	3E-04	TZ
contig01692	gi|67782245|gb|AAY81965.1| importin beta 1 [*Aplysia californica*] 48	3E-04	YU
contig03786	gi|145235872|ref|XP_001390584.1| unnamed protein product [*Asperg*... 49	2E-04	D
contig01624	gi|109829212|sp|P0C1J6|FKBP4_RHIOR FK506-binding protein 4 (Pept... 49	2E-04	O
contig05800	gi|114582838|ref|XP_001157111.1| PREDICTED: similar to KIAA2012 ... 48	2E-04	O
contig09522	gi|195573566|ref|XP_002104763.1| GD18276 [*Drosophila simulans*] >... 48	2E-04	O
contig07727	gi|118778060|ref|XP_308434.3| AGAP007398-PA [*Anopheles gambiae* s... 49	2E-04	OU
contig04133	gi|47228546|emb|CAG05366.1| unnamed protein product [*Tetraodon n*... 49	2E-04	T
contig11053	gi|169145619|emb|CAQ14613.1| notch homolog 2 [*Danio rerio*] 48	2E-04	T
contig01864	gi|195388476|ref|XP_002052906.1| GJ19592 [*Drosophila virilis*] >g... 48	2E-04	TZ

Contig	BLAST result	e value	Class
contig05518	gi|91077500|ref|XP_969314.1| PREDICTED: similar to exocyst compl... 48	2E-04	U
contig09437	gi|156548143|ref|XP_001606718.1| PREDICTED: similar to signal re... 49	2E-04	U
contig10659	gi|193584811|ref|XP_001949052.1| PREDICTED: similar to membrin [... 48	2E-04	U
contig10988	gi|194227296|ref|XP_001491209.2| PREDICTED: similar to acyl-Coen... 48	2E-04	U
contig01514	gi|195474225|ref|XP_002089392.1| GE19081 [*Drosophila yakuba*] >gn... 39	2E-04	V
contig10999	gi|110758166|ref|XP_623577.2| PREDICTED: similar to sallimus CG1... 52	2E-04	Z
contig05227	gi|194909250|ref|XP_001981914.1| GG12306 [*Drosophila erecta*] >gn... 50	1E-04	D
contig03672	gi|91091294|ref|XP_970147.1| PREDICTED: similar to AGAP001802-PA... 49	1E-04	O
contig03833	gi|28302342|gb|AAH46690.1| LOC398495 protein [*Xenopus laevis*] 49	1E-04	O
contig08335	gi|193641094|ref|XP_001948739.1| PREDICTED: similar to acylpepti... 49	1E-04	O
contig09574	gi|158286268|ref|XP_308650.4| AGAP007107-PA [*Anopheles gambiae* s... 49	1E-04	O
contig10138	gi|115783450|ref|XP_790411.2| PREDICTED: similar to Usp16 protei... 49	1E-04	O
contig10577	gi|66499725|ref|XP_397154.2| PREDICTED: similar to CG31915-PA [A... 49	1E-04	O
contig05376	gi|114606743|ref|XP_518409.2| PREDICTED: inositol 1,4,5-triphosp... 49	1E-04	T
contig07284	gi|115951942|ref|XP_001184423.1| PREDICTED: similar to alpha-2-m... 49	1E-04	T
contig04841	gi|149757279|ref|XP_001503441.1| PREDICTED: similar to longevity... 49	1E-04	U
contig08992	gi|195035974|ref|XP_001989446.1| GH18809 [*Drosophila grimshawi*] ... 44	1E-04	WT
contig06665	gi|193582389|ref|XP_001948175.1| PREDICTED: similar to short win... 49	1E-04	Z
contig07425	gi|195328617|ref|XP_002031011.1| GM24264 [*Drosophila sechellia*] ... 49	1E-04	Z
contig02695	gi|74096307|ref|NP_001027766.1| HR-29-like protein [*Ciona intest*... 50	8E-05	O
contig08120	gi|170571605|ref|XP_001891790.1| alpha-1,3-mannosyltransferase [... 50	7E-05	M
contig08789	gi|156546249|ref|XP_001605335.1| PREDICTED: similar to ubiquitin... 50	7E-05	O
contig04554	gi|168823548|ref|NP_001108403.1| hypothetical protein LOC1001413... 50	7E-05	U
contig02624	gi|91083805|ref|XP_973263.1| PREDICTED: similar to importin subu... 50	7E-05	YU
contig12249	gi|194679896|ref|XP_584352.4| PREDICTED: similar to inner centro... 50	6E-05	D
contig07713	gi|57530768|ref|NP_001006370.1| protein disulfide isomerase fami... 50	6E-05	O
contig05681	gi|156543770|ref|XP_001606220.1| PREDICTED: similar to CG7422-PB... 33	6E-05	T
contig11463	gi|119640054|gb|ABL85461.1| Wiskott-Aldrich syndrome protein [Su... 50	6E-05	TZ
contig12526	gi|91087669|ref|XP_973734.1| PREDICTED: similar to putative myos... 41	6E-05	Z
contig05375	gi|115629176|ref|XP_786317.2| PREDICTED: hypothetical protein [S... 47	5E-05	O
contig11054	gi|66500390|ref|XP_623298.1| PREDICTED: similar to Tropomyosin 1... 51	5E-05	Z
contig02176	gi|194741252|ref|XP_001953103.1| GF17600 [*Drosophila ananassae*] ... 50	4E-05	O
contig06912	gi|195446934|ref|XP_002070989.1| GK25553 [*Drosophila willistoni*]... 51	3E-05	T
contig08029	gi|123445530|ref|XP_001311524.1| conserved hypothetical protein ... 51	3E-05	T
contig07963	gi|862467|dbj|BAA06909.1| limulus intracellular coagulation inhi... 51	3E-05	V
contig08255	gi|126327982|ref|XP_001370272.1| PREDICTED: similar to facilitat... 51	3E-05	YU
contig11078	gi|47212600|emb|CAF93042.1| unnamed protein product [*Tetraodon n*... 51	3E-05	Z
contig08725	gi|156537388|ref|XP_001606740.1| PREDICTED: similar to cell divi... 52	2E-05	D
contig02274	gi|109497997|ref|XP_001070833.1| PREDICTED: hypothetical protein... 52	2E-05	O
contig03387	gi|56159969|gb|AAV80796.1| putative secreted salivary gland pept... 52	2E-05	O
contig10252	gi|119588531|gb|EAW68125.1| low density lipoprotein receptor cla... 54	2E-05	O
contig05389	gi|149064402|gb|EDM14605.1| casein kinase 1, alpha 1, isoform CR... 52	2E-05	T
contig10475	gi|148231426|ref|NP_001080095.1| growth hormone inducible transm... 52	2E-05	T
contig04419	gi|156545080|ref|XP_001601330.1| PREDICTED: similar to Nicastrin... 52	2E-05	TO
contig00812	gi|66518227|ref|XP_393502.2| PREDICTED: similar to CG10686-PA [A... 56	2E-05	U
contig05943	gi|157124457|ref|XP_001660469.1| low-density lipoprotein recepto... 52	1E-05	T
contig03751	gi|158299275|ref|XP_554113.3| AGAP010206-PA [*Anopheles gambiae* s... 40	1E-05	U
contig08782	gi|170037865|ref|XP_001846775.1| transport protein sec23 [*Culex* ... 52	1E-05	U
contig02333	gi|156549624|ref|XP_001603995.1| PREDICTED: similar to conserved... 52	1E-05	YU
contig09695	gi|91091320|ref|XP_971490.1| PREDICTED: similar to proteasome su... 53	9E-06	O
contig11600	gi|13774082|gb|AAK38169.1| cathepsin L-like [*Fasciola hepatica*] 53	9E-06	O

Contig	BLAST result	e value	Class
contig12188	gi|114051792|ref|NP_001039436.1| serine peptidase inhibitor, Kun... 53	9E-06	O
contig11075	gi|197103110|ref|NP_001126096.1| transmembrane BAX inhibitor mot... 53	9E-06	T
contig03610	gi|126179667|ref|YP_001047632.1| beta-Ig-H3/fasciclin [*Methanocu*... 53	7E-06	MW
contig04971	gi|148704620|gb|EDL36567.1| proteasome (prosome, macropain) subu... 53	7E-06	O
contig07427	gi|189515588|ref|XP_001922908.1| PREDICTED: similar to frizzy-re... 54	5E-06	DO
contig10282	gi|110766269|ref|XP_624567.2| PREDICTED: similar to fizzy CG4274... 54	5E-06	DO
contig00915	gi|170040091|ref|XP_001847845.1| maternal tudor protein [*Culex q*... 54	5E-06	T
contig02528	gi|189237968|ref|XP_001811946.1| PREDICTED: similar to CG8155 CG... 54	5E-06	T
contig03043	gi|195546839|ref|NP_001124248.1| hypothetical protein LOC562169 ... 54	5E-06	U
contig06655	gi|189237585|ref|XP_975030.2| PREDICTED: similar to importin alp... 54	5E-06	U
contig11757	gi|66560412|ref|XP_624981.1| PREDICTED: similar to SLIT-ROBO Rho... 54	5E-06	Z
contig11816	gi|33348836|gb|AAQ16118.1| cathepsin L-like cysteine proteinase ... 54	4E-06	O
contig09904	gi|158291648|ref|XP_313165.4| AGAP004246-PA [*Anopheles gambiae* s... 54	4E-06	T
contig12278	gi|149720830|ref|XP_001490387.1| PREDICTED: similar to RIO kinas... 54	4E-06	T
contig06011	gi|157120691|ref|XP_001659725.1| vacuolar protein sorting-associ... 54	4E-06	U
contig04861	gi|48118518|ref|XP_396430.1| PREDICTED: similar to CG14211-PB [A... 54	4E-06	V
contig05756	gi|115609883|ref|XP_795679.2| PREDICTED: similar to ANAPC2 prote... 48	3E-06	DO
contig09133	gi|114052713|ref|NP_001040543.1| proteasome 26S non-ATPase subun... 54	3E-06	O
contig04036	gi|158297644|ref|XP_317844.3| AGAP011463-PA [*Anopheles gambiae* s... 54	3E-06	T
contig06963	gi|82754297|gb|ABB89946.1| ace1 type acetylcholinesterase [*Blatt*... 54	3E-06	T
contig00742	gi|149010993|ref|ZP_01832298.1| argininosuccinate synthase [*Stre*... 57	3E-06	TV
contig02199	gi|190571419|ref|YP_001975777.1| signal recognition particle pro... 54	3E-06	U
contig06028	gi|47211513|emb|CAF90309.1| unnamed protein product [*Tetraodon n*... 54	3E-06	U
contig08380	gi|170044582|ref|XP_001849921.1| mitochondrial import receptor s... 54	3E-06	U
contig09366	gi|195441430|ref|XP_002068512.1| GK20379 [*Drosophila willistoni*]... 54	3E-06	Z
contig07043	gi|149755241|ref|XP_001495972.1| PREDICTED: zinc finger, CCHC do... 55	2E-06	D
contig08196	gi|148232744|ref|NP_001081724.1| wee1 homolog [*Xenopus laevis*] >... 55	2E-06	D
contig07585	gi|116055404|emb|CAL58072.1| polyubiquitin 2 (ISS) [*Ostreococcus*... 55	2E-06	DO
contig04062	gi|91091796|ref|XP_970426.1| PREDICTED: similar to dynactin P62 ... 55	2E-06	N
contig02757	gi|48113502|ref|XP_396350.1| PREDICTED: similar to Peptidylglyci... 55	2E-06	O
contig07025	gi|16768432|gb|AAL28435.1| GM04535p [*Drosophila melanogaster*] 55	2E-06	O
contig10511	gi|196009778|ref|XP_002114754.1| hypothetical protein TRIADDRAFT... 55	2E-06	YU
contig06162	gi|19114087|ref|NP_593175.1| actin binding protein, coronin Crn1... 55	2E-06	Z
contig01259	gi|91093707|ref|XP_967114.1| PREDICTED: similar to sex-determini... 55	1E-06	M
contig01257	gi|156401011|ref|XP_001639085.1| predicted protein [*Nematostella*... 56	1E-06	O
contig05898	gi|91077894|ref|XP_973141.1| PREDICTED: similar to predicted pro... 55	1E-06	O
contig07163	gi|91093697|ref|XP_966486.1| PREDICTED: similar to ADAM metallop... 53	1E-06	O
contig08800	gi|194883632|ref|XP_001975905.1| GG20306 [*Drosophila erecta*] >gn... 55	1E-06	T
contig05944	gi|126323512|ref|XP_001364194.1| PREDICTED: similar to adaptor-r... 55	1E-06	U
contig07178	gi|170036594|ref|XP_001846148.1| importin subunit beta [*Culex qu*... 55	1E-06	YU
contig11328	gi|74927078|sp|Q86GF7|CRUST_PANBO Crustapain precursor (NsCys) >... 56	8E-07	O
contig09675	gi|108998743|ref|XP_001096957.1| PREDICTED: similar to PTEN indu... 56	8E-07	T
contig12386	gi|25148442|ref|NP_741323.1| K08D12.3a [*Caenorhabditis elegans*] ... 40	6E-07	O
contig00781	gi|115638628|ref|XP_784181.2| PREDICTED: similar to high affinit... 57	6E-07	T
contig01475	gi|63259175|gb|AAY40292.1| replicase [Porcine circovirus 2] 57	6E-07	T
contig00329	gi|156398162|ref|XP_001638058.1| predicted protein [*Nematostella*... 57	6E-07	U
contig05873	gi|115720191|ref|XP_792773.2| PREDICTED: similar to Wu:fc30a11 p... 57	6E-07	U
contig05190	gi|28932704|gb|AAO60046.1| midgut cysteine proteinase 3 [*Rhipice*... 57	5E-07	O
contig09173	gi|194224024|ref|XP_001917420.1| PREDICTED: sema domain, seven t... 57	5E-07	T
contig10385	gi|156374335|ref|XP_001629763.1| predicted protein [*Nematostella*... 57	5E-07	T
contig02592	gi|156546934|ref|XP_001603964.1| PREDICTED: similar to outer mit... 57	5E-07	U

Contig	BLAST result	e value	Class
contig06405	gi|157118247|ref|XP_001653133.1| mbp-1 interacting protein-2a [*A*... 57	5E-07	U
contig11589	gi|157310311|emb|CAN43867.1| outer membrane protein 25b [*Brucell*... 57	4E-07	OR
contig04193	gi|195332648|ref|XP_002033009.1| GM21084 [*Drosophila sechellia*] ... 57	4E-07	T
contig04802	gi|194384828|dbj|BAG60820.1| unnamed protein product [*Homo sapiens*] 57	4E-07	TZ
contig02563	gi|156548151|ref|XP_001606762.1| PREDICTED: similar to microtubu... 57	4E-07	Z
contig08064	gi|110768018|ref|XP_623323.2| PREDICTED: similar to zipper CG157... 57	4E-07	Z
contig00694	gi|72043691|ref|XP_791396.1| PREDICTED: hypothetical protein [St... 58	3E-07	O
contig02704	gi|156384849|ref|XP_001633345.1| predicted protein [*Nematostella*... 60	3E-07	O
contig12714	gi|194212734|ref|XP_001502891.2| PREDICTED: ubiquitination facto... 59	2E-07	O
contig07417	gi|157104296|ref|XP_001648341.1| growth hormone inducible transm... 59	2E-07	T
contig08164	gi|148231247|ref|NP_001079938.1| proto-oncogene tyrosine-protein... 58	2E-07	T
contig08184	gi|115977127|ref|XP_001179234.1| PREDICTED: similar to Sip-prov ... 59	2E-07	T
contig09394	gi|66499366|ref|XP_393243.2| PREDICTED: similar to Syndapin CG33... 58	2E-07	TUZ
contig02944	gi|170047259|ref|XP_001851146.1| conserved hypothetical protein ... 58	2E-07	U
contig05691	gi|47214953|emb|CAG10775.1| unnamed protein product [*Tetraodon n*... 53	2E-07	U
contig05982	gi|110762380|ref|XP_391944.3| PREDICTED: similar to rhea CG6831-... 59	2E-07	Z
contig10304	gi|168055852|ref|XP_001779937.1| predicted protein [*Physcomitrel*... 58	2E-07	Z
contig02366	gi|71997077|ref|NP_001022985.1| Tubulin Tyrosine Ligase Like fam... 48	1E-07	O
contig10668	gi|20090338|ref|NP_616413.1| heat shock protein 40 [*Methanosarci*... 59	1E-07	O
contig02056	gi|15487298|dbj|BAB64539.1| natriuretic-peptide receptor A [*Rana*... 50	1E-07	T
contig09019	gi|157773297|ref|XP_001664713.1| hypothetical protein CBG09108 [... 59	1E-07	T
contig11613	gi|158286904|ref|XP_308996.3| AGAP006746-PA [*Anopheles gambiae* s... 60	1E-07	Z
contig03172	gi|163838684|ref|NP_001106225.1| ubiquinol-cytochrome c reductas... 59	9E-08	O
contig07671	gi|156351062|ref|XP_001622345.1| predicted protein [*Nematostella*... 59	9E-08	O
contig10596	gi|195133496|ref|XP_002011175.1| GI16393 [*Drosophila mojavensis*]... 59	9E-08	TU
contig10500	gi|156537586|ref|XP_001607692.1| PREDICTED: similar to Sorting n... 59	9E-08	ZUD
contig03869	gi|169646700|ref|NP_613056.2| zinc finger, CCHC domain containin... 60	7E-08	O
contig02825	gi|110760264|ref|XP_393691.3| PREDICTED: similar to Serine/threo... 60	7E-08	T
contig10409	gi|115742433|ref|XP_001198028.1| PREDICTED: similar to MGC80624 ... 60	7E-08	U
contig08971	gi|115647121|ref|XP_783397.2| PREDICTED: similar to phosphatidyl... 46	6E-08	MO
contig06588	gi|91083895|ref|XP_974479.1| PREDICTED: similar to ATPase family... 60	6E-08	O
contig10325	gi|83701157|gb|ABC41138.1| small heat shock protein p26 [*Artemia*... 60	6E-08	O
contig04318	gi|194225289|ref|XP_001494645.2| PREDICTED: similar to Fibulin 5... 60	6E-08	T
contig06047	gi|195575320|ref|XP_002105627.1| GD21583 [*Drosophila simulans*] >... 60	6E-08	U
contig08453	gi|148235839|ref|NP_001080187.1| p47 [*Xenopus laevis*] >gnl|BL_OR... 60	6E-08	Y
contig09181	gi|91083247|ref|XP_973990.1| PREDICTED: similar to O-fucosyltran... 60	5E-08	O
contig05180	gi|158301431|ref|XP_321122.4| AGAP001939-PA [*Anopheles gambiae* s... 60	5E-08	U
contig10350	gi|17540400|ref|NP_501169.1| F42C5.9 [*Caenorhabditis elegans*] >g... 60	5E-08	Z
contig08194	gi|110764542|ref|XP_395416.3| PREDICTED: similar to PCI domain c... 60	4E-08	D
contig11035	gi|50746643|ref|XP_420590.1| PREDICTED: similar to cyclin I [*Gal*... 60	4E-08	D
contig01120	gi|50400849|sp|P84032|PN16_PHONI Venom protein PN16C3 62	4E-08	MW
contig03262	gi|170577734|ref|XP_001894118.1| Cathepsin L-like precursor [*Bru*... 60	4E-08	O
contig04169	gi|29841011|gb|AAP06024.1| SJCHGC09407 protein [*Schistosoma japo*... 60	4E-08	O
contig07149	gi|160774139|gb|AAI55471.1| Unknown (protein for IMAGE:7725043) ... 60	4E-08	O
contig10922	gi|60302732|ref|NP_001012558.1| tubulin tyrosine ligase-like fam... 60	4E-08	O
contig10445	gi|170047146|ref|XP_001851095.1| meiotic checkpoint regulator cu... 61	3E-08	DO
contig09690	gi|47211865|emb|CAF95367.1| unnamed protein product [*Tetraodon n*... 61	3E-08	DZ
contig07900	gi|170571908|ref|XP_001891914.1| ThiF family protein [*Brugia mal*... 61	3E-08	O
contig09514	gi|194755154|ref|XP_001959857.1| GF13077 [*Drosophila ananassae*] ... 61	3E-08	O
contig09984	gi|195427169|ref|XP_002061651.1| GK17108 [*Drosophila willistoni*]... 61	3E-08	O
contig02348	gi|159570264|emb|CAP19571.1| signal transducing adaptor molecule... 61	3E-08	T

Contig	BLAST result	e value	Class
contig05377	gi|170073474|ref|XP_001870381.1| tomosyn [*Culex quinquefasciatus*... 61	3E-08	U
contig08101	gi|91092748|ref|XP_973348.1| PREDICTED: similar to pyrazinamidas... 61	3E-08	V
contig08959	gi|189233780|ref|XP_001814557.1| PREDICTED: similar to Kinesin-7... 61	3E-08	Z
contig09244	gi|156538078|ref|XP_001607596.1| PREDICTED: similar to beta chai... 61	3E-08	Z
contig08097	gi|193659754|ref|XP_001946896.1| PREDICTED: similar to mystery 4... 62	2E-08	DZ
contig03004	gi|196476658|gb|ACG76195.1| alpha-2-macroglobulin [*Amblyomma ame*... 62	2E-08	O
contig03114	gi|34539117|gb|AAQ74442.1| glutathione S-transferase [*Rhipicepha*... 61	2E-08	O
contig04479	gi|47219312|emb|CAG10941.1| unnamed protein product [*Tetraodon n*... 42	2E-08	O
contig09370	gi|164655843|ref|XP_001729050.1| hypothetical protein MGL_3838 [... 62	2E-08	O
contig10245	gi|149571552|ref|XP_001518754.1| PREDICTED: similar to Protein p... 62	2E-08	OT
contig06815	gi|45361393|ref|NP_989274.1| protein phosphatase 2 (formerly 2A)... 62	2E-08	T
contig00314	gi|189241696|ref|XP_970104.2| PREDICTED: similar to LIM protein ... 61	2E-08	TZ
contig05858	gi|91089831|ref|XP_969724.1| PREDICTED: similar to formin 1,2/ca... 61	2E-08	TZ
contig10790	gi|156391901|ref|XP_001635788.1| predicted protein [*Nematostella*... 62	1E-08	D
contig11423	gi|194755108|ref|XP_001959834.1| GF13064 [*Drosophila ananassae*] ... 62	1E-08	D
contig02943	gi|163843912|ref|YP_001628316.1| O-sialoglycoprotein endopeptida... 62	1E-08	O
contig07367	gi|118085702|ref|XP_418609.2| PREDICTED: similar to dnaJ-like pr... 62	1E-08	O
contig11634	gi|149032841|gb|EDL87696.1| rCG42109, isoform CRA_a [*Rattus norv*... 62	1E-08	O
contig12028	gi|170573427|ref|XP_001892466.1| hypothetical protein Bm1_04940 ... 62	1E-08	O
contig00552	gi|115702407|ref|XP_001177220.1| PREDICTED: similar to LOC615074... 62	1E-08	T
contig00758	gi|91079328|ref|XP_968347.1| PREDICTED: similar to AGAP009901-PA... 62	1E-08	T
contig07052	gi|66517761|ref|XP_392905.2| PREDICTED: similar to cAMP-dependen... 62	1E-08	T
contig07729	gi|62466117|gb|AAX83388.1| Sds [*Schistosoma mansoni*] 62	1E-08	T
contig06206	gi|170033357|ref|XP_001844544.1| conserved hypothetical protein ... 62	1E-08	U
contig12279	gi|22164300|gb|AAM93655.1|AF483733_1 putative 4.3 kDa secreted p... 62	1E-08	U
contig08548	gi|114153202|gb|ABI52767.1| serpin [*Argas monolakensis*] 62	1E-08	V
contig01414	gi|114153294|gb|ABI52813.1| GGY domain protein [*Argas monolakensis*] 64	1E-08	YU
contig07301	gi|6682319|emb|CAB64662.1| myosin heavy chain [*Mytilus galloprov*... 62	1E-08	Z
contig04829	gi|91081971|ref|XP_967978.1| PREDICTED: similar to tumor endothe... 63	8E-09	W
contig06326	gi|114864933|gb|ABI83786.1| Arp2/3 complex subunit ARPC5 [*Anophe*... 63	8E-09	Z
contig02882	gi|193596505|ref|XP_001945593.1| PREDICTED: similar to 26S prote... 63	7E-09	O
contig06458	gi|157813218|gb|ABV81354.1| putative 26S proteasome non-ATPase r... 63	7E-09	O
contig11935	gi|118638258|gb|ABL09302.1| allergen Aca s 8 [*Acarus siro*] 64	7E-09	O
contig08441	gi|197692443|dbj|BAG70185.1| protein phosphatase 1A isoform 1 [H... 63	7E-09	T
contig06303	gi|195388318|ref|XP_002052827.1| GJ19747 [*Drosophila virilis*] >g... 63	7E-09	U
contig01803	gi|2570145|dbj|BAA22991.1| cyclin A [*Hemicentrotus pulcherrimus*] 66	5E-09	D
contig00286	gi|48138650|ref|XP_393416.1| PREDICTED: similar to Downstream of... 64	5E-09	T
contig07954	gi|170062766|ref|XP_001866812.1| integrin-linked protein kinase ... 64	5E-09	T
contig01738	gi|33440467|gb|AAH56211.1| Platelet-activating factor acetylhydr... 64	5E-09	TV
contig08910	gi|197322816|gb|ACH69159.1| myosin heavy chain [*Bombyx mori*] 64	5E-09	Z
contig02955	gi|193606239|ref|XP_001950033.1| PREDICTED: similar to anaphase ... 64	4E-09	DO
contig01310	gi|33667928|gb|AAQ24541.1| Blo t 1 allergen [*Blomia tropicalis*] 64	4E-09	O
contig02815	gi|72030199|ref|XP_780027.1| PREDICTED: similar to MGC80929 prot... 64	4E-09	O
contig06695	gi|167518423|ref|XP_001743552.1| predicted protein [*Monosiga bre*... 64	4E-09	U
contig08012	gi|115655549|ref|XP_789920.2| PREDICTED: similar to CG1599-PA [*S*... 64	4E-09	U
contig06980	gi|156544772|ref|XP_001606200.1| PREDICTED: similar to ubiquitin... 64	3E-09	O
contig09009	gi|114053293|ref|NP_001039853.1| heat shock 70kDa protein 14 [*Bo*... 64	3E-09	O
contig09576	gi|196000510|ref|XP_002110123.1| hypothetical protein TRIADDRAFT... 64	3E-09	O
contig03319	gi|170046387|ref|XP_001850749.1| peroxisomal biogenesis factor 1... 64	3E-09	U
contig07895	gi|50513239|gb|AAT77811.1| calpain B [*Gecarcinus lateralis*] 58	2E-09	OT
contig02303	gi|91078242|ref|XP_970298.1| PREDICTED: similar to 1-phosphatidy... 65	2E-09	T

Contig	BLAST result	e value	Class
contig06095	gi|156543205|ref|XP_001606305.1| PREDICTED: similar to SD02996p ... 65	2E-09	T
contig07452	gi|156366264|ref|XP_001627059.1| predicted protein [*Nematostella*... 65	2E-09	T
contig07748	gi|149714295|ref|XP_001504277.1| PREDICTED: similar to Fas apopt... 65	2E-09	T
contig08601	gi|91092436|ref|XP_968632.1| PREDICTED: similar to laminin gamma... 65	2E-09	W
contig09125	gi|158297035|ref|XP_317338.4| AGAP008118-PA [*Anopheles gambiae* s... 65	1E-09	D
contig01568	gi|121543943|gb|ABM55636.1| putative apolipoprotein D [*Maconelli*... 67	1E-09	M
contig03969	gi|47222375|emb|CAG05124.1| unnamed protein product [*Tetraodon n*... 65	1E-09	O
contig04104	gi|115668711|ref|XP_791717.2| PREDICTED: similar to conserved hy... 66	1E-09	O
contig05360	gi|139000435|dbj|BAF51711.1| tick legumain [*Haemaphysalis longic*... 65	1E-09	O
contig05941	gi|195384621|ref|XP_002051013.1| GJ22461 [*Drosophila virilis*] >g... 66	1E-09	O
contig06104	gi|91078536|ref|XP_970577.1| PREDICTED: similar to ganglioside-i... 65	1E-09	O
contig07738	gi|156543233|ref|XP_001606546.1| PREDICTED: similar to ENSANGP00... 65	1E-09	O
contig11776	gi|168003405|ref|XP_001754403.1| predicted protein [*Physcomitrel*... 66	1E-09	O
contig01722	gi|189233683|ref|XP_970087.2| PREDICTED: similar to Delta [*Tribo*... 66	1E-09	T
contig05547	gi|83272632|gb|ABC00801.1| LOC402870-like protein [*Pomacentrus m*... 65	1E-09	T
contig06390	gi|195050911|ref|XP_001992994.1| GH13339 [*Drosophila grimshawi*] ... 65	1E-09	Z
contig08044	gi|148725190|emb|CAN88398.1| novel protein (zgc:56161) [*Danio re*... 66	8E-10	D
contig06652	gi|156365518|ref|XP_001626692.1| predicted protein [*Nematostella*... 66	8E-10	O
contig07811	gi|170591296|ref|XP_001900406.1| Coronin-like protein [*Brugia m*a... 66	8E-10	Z
contig11630	gi|193643604|ref|XP_001943670.1| PREDICTED: similar to predicted... 67	6E-10	O
contig11170	gi|144925078|gb|ABP03876.1| Hly-III related proteins [*Medicago t*... 67	6E-10	RT
contig02246	gi|91080341|ref|XP_974659.1| PREDICTED: similar to GA19895-PA [*T*... 67	6E-10	T
contig06908	gi|189237088|ref|XP_969702.2| PREDICTED: similar to GA17645-PA [... 67	6E-10	T
contig07905	gi|156384880|ref|XP_001633360.1| predicted protein [*Nematostella*... 67	6E-10	YU
contig06168	gi|189234407|ref|XP_975060.2| PREDICTED: similar to myosin vi [*T*... 67	5E-10	Z
contig09471	gi|157133635|ref|XP_001662951.1| endoplasmin [*Aedes aegypti*] >gn... 67	3E-10	O
contig10492	gi|193613058|ref|XP_001952614.1| PREDICTED: similar to Y48C3A.16... 67	3E-10	O
contig10658	gi|41054301|ref|NP_956052.1| ariadne ubiquitin-conjugating enzym... 68	3E-10	O
contig07749	gi|189523269|ref|XP_687578.3| PREDICTED: similar to Rap guanine ... 68	3E-10	T
contig10263	gi|189234212|ref|XP_970946.2| PREDICTED: similar to adducin [*Tri*... 68	3E-10	TZ
contig03007	gi|156383838|ref|XP_001633039.1| predicted protein [*Nematostella*... 67	3E-10	U
contig07411	gi|193664496|ref|XP_001943041.1| PREDICTED: similar to short sto... 68	3E-10	Z
contig00703	gi|159155128|gb|AAI54789.1| Proteasome (prosome, macropain) 26S ... 68	2E-10	O
contig04640	gi|66546020|ref|XP_623947.1| PREDICTED: similar to Probable 26S ... 68	2E-10	O
contig11458	gi|156543740|ref|XP_001605962.1| PREDICTED: similar to tripeptid... 70	2E-10	O
contig08039	gi|156384222|ref|XP_001633230.1| predicted protein [*Nematostella*... 69	2E-10	OW
contig07989	gi|165972429|ref|NP_001107093.1| hypothetical protein LOC570768 ... 68	2E-10	TR
contig02431	gi|158287103|ref|XP_309162.4| AGAP000969-PA [*Anopheles gambiae* s... 65	2E-10	Z
contig06634	gi|91085145|ref|XP_966691.1| PREDICTED: similar to choline/ethan... 69	1E-10	M
contig03937	gi|146163744|ref|XP_001471259.1| cathepsin z [*Tetrahymena thermo*... 69	1E-10	O
contig11629	gi|189234978|ref|XP_001807535.1| PREDICTED: similar to lysosomal... 69	1E-10	O
contig02754	gi|91089445|ref|XP_966341.1| PREDICTED: similar to rho guanine d... 69	1E-10	T
contig03763	gi|193662043|ref|XP_001949462.1| PREDICTED: similar to proto-onc... 69	1E-10	T
contig06847	gi|156550147|ref|XP_001606081.1| PREDICTED: similar to ENSANGP00... 69	9E-11	M
contig07760	gi|193787078|dbj|BAG51901.1| unnamed protein product [*Homo sapiens*] 69	9E-11	M
contig01285	gi|156545036|ref|XP_001600401.1| PREDICTED: similar to conserved... 69	9E-11	O
contig08237	gi|158285616|ref|XP_308398.3| AGAP007474-PA [*Anopheles gambiae* s... 69	9E-11	Z
contig00710	gi|91090252|ref|XP_969934.1| PREDICTED: similar to maggie CG1498... 70	8E-11	U
contig09487	gi|170033703|ref|XP_001844716.1| cell division protease ftsH [*Cu*... 70	7E-11	O
contig04834	gi|196006640|ref|XP_002113186.1| hypothetical protein TRIADDRAFT... 70	7E-11	T
contig01582	gi|158711988|gb|ABW79798.1| vitellogenin receptor [*Penaeus monodon*] 71	6E-11	T

Contig	BLAST result	e value	Class
contig11498	gi|197129867|gb|ACH46365.1| putative septin 11 [*Taeniopygia gutt*... 70	4E-11	D
contig07756	gi|156365829|ref|XP_001626845.1| predicted protein [*Nematostella*... 70	4E-11	O
contig07432	gi|91091170|ref|XP_971489.1| PREDICTED: similar to huntingtin in... 59	3E-11	Z
contig05905	gi|156552545|ref|XP_001600397.1| PREDICTED: similar to protein a... 71	2E-11	D
contig05969	gi|156379089|ref|XP_001631291.1| predicted protein [*Nematostella*... 72	2E-11	O
contig10950	gi|47085937|ref|NP_998328.1| autocrine motility factor receptor ... 52	2E-11	O
contig08458	gi|149052895|gb|EDM04712.1| rCG33182, isoform CRA_b [*Rattus norv*... 71	2E-11	OR
contig01714	gi|66525845|ref|XP_623057.1| PREDICTED: similar to CG5315-PA, is... 71	2E-11	RT
contig06810	gi|156376991|ref|XP_001630641.1| predicted protein [*Nematostella*... 72	2E-11	T
contig07112	gi|194208555|ref|XP_001490275.2| PREDICTED: similar to vacuolar ... 72	2E-11	U
contig10302	gi|158291370|ref|XP_312883.3| AGAP003185-PA [*Anopheles gambiae* s... 71	2E-11	U
contig01024	gi|157116832|ref|XP_001652865.1| nsfl1 cofactor P47 [*Aedes aegyp*... 74	2E-11	Y
contig10714	gi|198150846|gb|EAL29947.2| GA21581 [*Drosophila pseudoobscura* ps... 72	2E-11	Z
contig04804	gi|170038740|ref|XP_001847206.1| phospholipid scramblase 1 [*Cule*... 72	1E-11	M
contig06488	gi|195481167|ref|XP_002101542.1| GE15568 [*Drosophila yakuba*] >gn... 72	1E-11	O
contig09209	gi|170033167|ref|XP_001844450.1| ribosomal protein S6 kinase [*Cu*... 72	1E-11	RT
contig07647	gi|66561016|ref|XP_392778.2| PREDICTED: similar to CG40494-PA.3 ... 72	1E-11	T
contig00517	gi|114153200|gb|ABI52766.1| Defensin [*Argas monolakensis*] 72	1E-11	TR
contig08079	gi|150416761|gb|ABR68850.1| synaptotagmin [*Platynereis dumerilii*] 72	1E-11	TU
contig09042	gi|115608248|ref|XP_779975.2| PREDICTED: hypothetical protein [S... 72	1E-11	U
contig09384	gi|115947191|ref|XP_784884.2| PREDICTED: similar to karyopherin ... 72	1E-11	U
contig10022	gi|157813190|gb|ABV81340.1| putative signal recognition particle... 72	1E-11	U
contig02149	gi|156544131|ref|XP_001605898.1| PREDICTED: similar to ENSANGP00... 72	1E-11	Z
contig02939	gi|71896827|ref|NP_001025946.1| COP9 complex subunit 7a [*Gallus* ... 56	9E-12	OT
contig02089	gi|157093349|gb|ABV22329.1| ubiquitin-conjugating enzyme E2, cat... 73	8E-12	O
contig10665	gi|91089713|ref|XP_974951.1| PREDICTED: similar to ubiquitin spe... 73	8E-12	O
contig05394	gi|148233314|ref|NP_001087108.1| ubiquilin 2 [*Xenopus laevis*] >g... 73	8E-12	OR
contig11410	gi|55785689|dbj|BAD72613.1| granulin-like peptide precursor [*Miz*... 73	8E-12	T
contig09088	gi|149640886|ref|XP_001512501.1| PREDICTED: similar to Exportin ... 73	8E-12	YU
contig10056	gi|82698821|gb|ABB89211.1| troponin I protein [*Rhipicephalus hae*... 73	8E-12	Z
contig00292	gi|115748979|ref|XP_784731.2| PREDICTED: similar to Syvn1 protei... 73	6E-12	O
contig10116	gi|193580107|ref|XP_001944348.1| PREDICTED: similar to tep3, par... 73	6E-12	O
contig04781	gi|158289383|ref|XP_311125.4| AGAP000037-PA [*Anopheles gambiae* s... 74	5E-12	T
contig10692	gi|196012014|ref|XP_002115870.1| hypothetical protein TRIADDRAFT... 74	5E-12	U
contig02693	gi|91081971|ref|XP_967978.1| PREDICTED: similar to tumor endothe... 74	5E-12	W
contig05209	gi|58385719|ref|XP_314156.2| AGAP005246-PD [*Anopheles gambiae* st... 75	4E-12	V
contig03620	gi|119615295|gb|EAW94889.1| capping protein (actin filament) mus... 75	4E-12	Z
contig09938	gi|189241293|ref|XP_975097.2| PREDICTED: similar to conserved hy... 74	3E-12	O
contig10082	gi|126305110|ref|XP_001362347.1| PREDICTED: hypothetical protein... 74	3E-12	O
contig06999	gi|156547498|ref|XP_001605935.1| PREDICTED: similar to ENSANGP00... 50	3E-12	OT
contig04160	gi|193783717|dbj|BAG53822.1| unnamed protein product [*Homo sapiens*] 74	3E-12	TZ
contig10216	gi|193713573|ref|XP_001950161.1| PREDICTED: similar to AGAP00448... 74	3E-12	V
contig04851	gi|198145466|gb|EAL32378.2| GA14896 [*Drosophila pseudoobscura* ps... 75	2E-12	O
contig05743	gi|91080775|ref|XP_968281.1| PREDICTED: similar to rab gdp-disso... 75	2E-12	O
contig06107	gi|67083917|gb|AAY66893.1| SCF ubiquitin ligase complex [*Ixodes* ... 75	2E-12	O
contig10559	gi|195123633|ref|XP_002006308.1| GI18640 [*Drosophila mojavensis*]... 75	2E-12	O
contig02018	gi|156366264|ref|XP_001627059.1| predicted protein [*Nematostella*... 76	2E-12	T
contig03283	gi|115815405|ref|XP_794440.2| PREDICTED: hypothetical protein [*S*... 75	2E-12	T
contig07922	gi|161661029|gb|ABX75381.1| cysteine and glycine-rich protein [*L*... 75	2E-12	TZ
contig00597	gi|121309135|dbj|BAF43802.1| galectin [*Ornithodoros moubata*] >gn... 76	2E-12	W
contig05816	gi|193587184|ref|XP_001946129.1| PREDICTED: similar to beta chai... 75	2E-12	Z

Contig	BLAST result	e value	Class
contig01204	gi|163869040|ref|YP_001610271.1| chaperonin GroEL [*Bartonella tr*... 75	1E-12	O
contig07787	gi|17563052|ref|NP_503322.1| R09E12.3 [*Caenorhabditis elegans*] >... 76	1E-12	O
contig03567	gi|126331533|ref|XP_001377752.1| PREDICTED: hypothetical protein... 76	1E-12	TU
contig10123	gi|194380362|dbj|BAG63948.1| unnamed protein product [*Homo sapiens*] 75	1E-12	TV
contig08965	gi|118780654|ref|XP_310308.5| AGAP003769-PA [*Anopheles gambiae* s... 75	1E-12	YU
contig12098	gi|112143898|gb|ABI13158.1| putative cysteine protease [*Emiliani*... 76	7E-13	O
contig09790	gi|66472542|ref|NP_001018438.1| phosphatidylinositol-4-phosphate... 76	7E-13	T
contig04045	gi|72112336|ref|XP_789626.1| PREDICTED: hypothetical protein [*St*... 76	7E-13	TR
contig11023	gi|189536402|ref|XP_700411.3| PREDICTED: si:dkeyp-1e8.1 [*Danio r*... 76	7E-13	TR
contig03061	gi|157428048|ref|NP_001098932.1| ATPase family, AAA domain conta... 77	6E-13	O
contig03711	gi|115377235|ref|ZP_01464446.1| maleylacetoacetate isomerase [*St*... 77	6E-13	O
contig05834	gi|91079624|ref|XP_967576.1| PREDICTED: similar to dodo [*Triboli*... 77	6E-13	O
contig11157	gi|156537009|ref|XP_001608297.1| PREDICTED: similar to prefoldin... 77	6E-13	O
contig13341	gi|195439884|ref|XP_002067789.1| GK12524 [*Drosophila willistoni*]... 77	6E-13	U
contig01683	gi|71896055|ref|NP_001025616.1| pcmt1 protein [*Xenopus tropicali*... 77	4E-13	O
contig01372	gi|156400070|ref|XP_001638823.1| predicted protein [*Nematostella*... 80	4E-13	T
contig00466	gi|74096057|ref|NP_001027676.1| sec61 protein [*Ciona intestinali*... 64	4E-13	U
contig01544	gi|156553544|ref|XP_001601721.1| PREDICTED: similar to sorting n... 77	4E-13	U
contig09214	gi|156553544|ref|XP_001601721.1| PREDICTED: similar to sorting n... 77	4E-13	U
contig04898	gi|91089973|ref|XP_973833.1| PREDICTED: similar to Exportin 1, C... 77	4E-13	YU
contig02910	gi|158297830|ref|XP_318008.4| AGAP004801-PA [*Anopheles gambiae* s... 77	4E-13	Z
contig01596	gi|195023701|ref|XP_001985734.1| GH20962 [*Drosophila grimshawi*] ... 78	3E-13	DR
contig01091	gi|196008825|ref|XP_002114278.1| hypothetical protein TRIADDRAFT... 78	3E-13	DZ
contig05220	gi|148236500|ref|NP_001083999.1| similar to proteasome (prosome,... 77	3E-13	O
contig06212	gi|54261597|gb|AAH84600.1| LOC495281 protein [*Xenopus laevis*] 78	3E-13	O
contig09454	gi|126343155|ref|XP_001372015.1| PREDICTED: similar to inhibitor... 77	3E-13	OT
contig02577	gi|194318571|gb|ACF47675.1| guanine nucleotide-binding protein a... 77	3E-13	T
contig06706	gi|193580220|ref|XP_001945916.1| PREDICTED: similar to myotonin-... 77	3E-13	T
contig10622	gi|189235622|ref|XP_970209.2| PREDICTED: similar to map kinase-i... 78	3E-13	T
contig01214	gi|91087807|ref|XP_967674.1| PREDICTED: similar to vacuolar prot... 78	3E-13	U
contig02369	gi|157124488|ref|XP_001654070.1| ran-binding protein [*Aedes aegy*... 78	3E-13	U
contig08216	gi|66549728|ref|XP_392990.2| PREDICTED: similar to ADP ribosylat... 78	3E-13	U
contig11349	gi|72077602|ref|XP_797023.1| PREDICTED: similar to serpin 1 prec... 77	3E-13	V
contig02362	gi|195443760|ref|XP_002069562.1| GK11508 [*Drosophila willistoni*]... 77	3E-13	YU
contig02189	gi|170031478|ref|XP_001843612.1| tep3 [*Culex quinquefasciatus*] >... 78	2E-13	O
contig03901	gi|11079190|dbj|BAB17604.1| peroxiredoxin [*Haemaphysalis longico*... 78	2E-13	O
contig05892	gi|91087035|ref|XP_974421.1| PREDICTED: similar to synoviolin [*T*... 78	2E-13	O
contig02162	gi|170029149|ref|XP_001842456.1| tyrosine-protein kinase btk29a ... 78	2E-13	T
contig04164	gi|170030314|ref|XP_001843034.1| mystery 45A [*Culex quinquefasci*... 59	1E-13	DZ
contig03413	gi|156395766|ref|XP_001637281.1| predicted protein [*Nematostella*... 60	1E-13	O
contig09305	gi|158293614|ref|XP_314956.4| AGAP008822-PA [*Anopheles gambiae* s... 55	1E-13	O
contig05148	gi|197128799|gb|ACH45297.1| putative RAB14 member RAS oncogene f... 67	1E-13	U
contig10998	gi|161671318|gb|ABX75511.1| myosin light polypeptide 6 [*Lycosa s*... 79	1E-13	Z
contig09141	gi|62857965|ref|NP_001016573.1| hypothetical protein LOC549327 [... 79	9E-14	D
contig03006	gi|115951487|ref|XP_001189418.1| PREDICTED: similar to KIAA0369 ... 80	7E-14	DZ
contig00065	gi|170031478|ref|XP_001843612.1| tep3 [*Culex quinquefasciatus*] >... 80	7E-14	O
contig07773	gi|197129911|gb|ACH46409.1| putative NSFL1 cofactor [*Taeniopygia*... 80	7E-14	Y
contig09981	gi|156541807|ref|XP_001602555.1| PREDICTED: similar to GH01576p ... 80	5E-14	YU
contig08936	gi|156547617|ref|XP_001603638.1| PREDICTED: similar to ATP-depen... 80	4E-14	O
contig04168	gi|156549579|ref|XP_001602959.1| PREDICTED: similar to calpain B... 80	4E-14	OT
contig02838	gi|149408666|ref|XP_001509828.1| PREDICTED: similar to mitogen-a... 52	4E-14	T

Contig	BLAST result	e value	Class
contig05563	gi|139949068|ref|NP_001077235.1| protein tyrosine phosphatase ty... 80	4E-14	T
contig03016	gi|108742460|gb|ABG01866.1| putative accessory gland protein [Gr... 54	3E-14	O
contig04354	gi|193594276|ref|XP_001945478.1| PREDICTED: similar to proteasom... 81	3E-14	O
contig04389	gi|170579699|ref|XP_001894945.1| Ulp1 protease family, C-termina... 81	3E-14	O
contig00754	gi|195028748|ref|XP_001987238.1| GH21807 [*Drosophila grimshawi*] ... 82	3E-14	V
contig00964	gi|195391043|ref|XP_002054175.1| GJ24294 [*Drosophila virilis*] >g... 81	2E-14	D
contig05885	gi|156544752|ref|XP_001606012.1| PREDICTED: similar to ENSANGP00... 81	2E-14	DO
contig02323	gi|195539750|gb|AAI68042.1| Unknown (protein for MGC:185359) [*Xe*... 81	2E-14	O
contig07964	gi|170064267|ref|XP_001867454.1| conserved hypothetical protein ... 82	2E-14	O
contig11545	gi|21263742|sp|Q9I8F9|HSP71_ORYLA Heat shock 70 kDa protein 1 (H... 82	2E-14	O
contig04245	gi|41053487|ref|NP_956984.1| hypothetical protein LOC393663 [*Dan*... 81	2E-14	U
contig06827	gi|91086141|ref|XP_969127.1| PREDICTED: similar to protein trans... 82	2E-14	U
contig01466	gi|66508940|ref|XP_397201.2| PREDICTED: similar to CG6459-PA [Ap... 83	2E-14	V
contig02380	gi|194219245|ref|XP_001490868.2| PREDICTED: similar to ubiquitin... 82	1E-14	O
contig04680	gi|124487744|gb|ABN11959.1| putative chaperonin subunit 6a zeta ... 82	1E-14	O
contig06472	gi|91077894|ref|XP_973141.1| PREDICTED: similar to predicted pro... 82	1E-14	O
contig04497	gi|156537007|ref|XP_001608296.1| PREDICTED: similar to conserved... 82	1E-14	OT
contig08448	gi|115670856|ref|XP_781475.2| PREDICTED: hypothetical protein [*S*... 82	1E-14	T
contig01409	gi|156546010|ref|XP_001607952.1| PREDICTED: similar to conserved... 82	1E-14	Z
contig01807	gi|66513484|ref|XP_395374.2| PREDICTED: similar to CG5871-PA [*Ap*... 83	8E-15	O
contig07583	gi|151301141|ref|NP_001093092.1| proteasome beta subunit [*Bombyx*... 83	8E-15	O
contig01384	gi|115713356|ref|XP_001204091.1| PREDICTED: similar to glycine t... 83	8E-15	T
contig02931	gi|34850473|dbj|BAC87840.1| Armadillo [*Achaearanea tepidariorum*] 83	8E-15	TZ
contig05528	gi|115700014|ref|XP_791516.2| PREDICTED: hypothetical protein [*S*... 83	6E-15	U
contig06678	gi|56267941|gb|AAV85443.1| reverse transcriptase-like protein [*A*... 83	6E-15	U
contig11012	gi|149639872|ref|XP_001510959.1| PREDICTED: hypothetical protein... 86	5E-15	T
contig02947	gi|118094562|ref|XP_422476.2| PREDICTED: similar to zinc finger,... 84	4E-15	D
contig01602	gi|195389480|ref|XP_002053404.1| GJ23355 [*Drosophila virilis*] >g... 84	4E-15	O
contig07872	gi|153008177|ref|YP_001369392.1| ATP-dependent protease ATP-bind... 84	4E-15	O
contig08037	gi|154338918|ref|XP_001565681.1| hypothetical protein LbrM25_V2.... 57	3E-15	O
contig05510	gi|71896903|ref|NP_001025928.1| heat shock 70kD protein binding ... 84	3E-15	OT
contig00395	gi|47207591|emb|CAG02332.1| unnamed protein product [*Tetraodon n*... 84	3E-15	T
contig11190	gi|170037319|ref|XP_001846506.1| TBC1 domain family [*Culex quinq*... 84	3E-15	T
contig10136	gi|189241310|ref|XP_001815658.1| PREDICTED: similar to AGAP00480... 84	3E-15	TZ
contig04258	gi|91083677|ref|XP_968528.1| PREDICTED: similar to dynein light ... 85	2E-15	N
contig01045	gi|193596448|ref|XP_001951792.1| PREDICTED: similar to Hsc70Cb C... 86	2E-15	O
contig05048	gi|156401011|ref|XP_001639085.1| predicted protein [*Nematostella*... 85	2E-15	O
contig06558	gi|170064323|ref|XP_001867478.1| brain tumor protein [*Culex quin*... 85	2E-15	O
contig10237	gi|156551295|ref|XP_001601373.1| PREDICTED: similar to ENSANGP00... 85	2E-15	O
contig02748	gi|194218564|ref|XP_001917008.1| PREDICTED: low density lipoprot... 87	2E-15	T
contig07826	gi|31198799|ref|XP_308347.1| AGAP007532-PA [*Anopheles gambiae* st... 85	2E-15	W
contig03409	gi|23491588|dbj|BAC16746.1| myosin heavy chain [*Branchiostoma be*... 85	2E-15	Z
contig03562	gi|161669254|gb|ABX75479.1| myosin heavy chain [*Lycosa singorien*... 85	2E-15	Z
contig01519	gi|195588777|ref|XP_002084134.1| GD14099 [*Drosophila simulans*] >... 86	1E-15	O
contig03414	gi|149898803|gb|ABR27863.1| Sec61 protein translocation complex ... 87	1E-15	O
contig06455	gi|148231811|ref|NP_001080017.1| hypothetical protein LOC379708 ... 86	1E-15	TW
contig09761	gi|156547359|ref|XP_001603094.1| PREDICTED: similar to ENSANGP00... 86	9E-16	O
contig12144	gi|50734130|ref|XP_418980.1| PREDICTED: similar to Leukocyte ela... 87	8E-16	V
contig02417	gi|47206961|emb|CAF90782.1| unnamed protein product [*Tetraodon n*... 88	7E-16	O
contig04028	gi|115638630|ref|XP_790132.2| PREDICTED: similar to GABA neurotr... 86	7E-16	T
contig12163	gi|146217392|gb|ABQ10738.1| cathepsin D [*Penaeus monodon*] 87	6E-16	O

Contig	BLAST result	e value	Class
contig03158	gi|47218173|emb|CAG10093.1| unnamed protein product [*Tetraodon n*... 87	6E-16	U
contig05986	gi|72091589|ref|XP_791815.1| PREDICTED: hypothetical protein [*St*... 87	5E-16	OR
contig06442	gi|193707017|ref|XP_001952867.1| PREDICTED: similar to septin [*A*... 87	4E-16	DZU
contig07741	gi|47206576|emb|CAF94013.1| unnamed protein product [*Tetraodon n*... 87	4E-16	O
contig04458	gi|156542512|ref|XP_001600274.1| PREDICTED: similar to glucosida... 87	4E-16	T
contig05410	gi|148222958|ref|NP_001085021.1| hypothetical protein LOC432085 ... 87	3E-16	O
contig02941	gi|156538200|ref|XP_001601553.1| PREDICTED: similar to GA10497-P... 87	3E-16	T
contig11338	gi|157106589|ref|XP_001649392.1| coatomer beta subunit [*Aedes ae*... 87	3E-16	U
contig01157	gi|162415911|gb|ABX89274.1| serpin peptidase inhibitor, clade B,... 87	3E-16	V
contig07835	gi|110756556|ref|XP_396810.3| PREDICTED: similar to Nucleoporin ... 87	3E-16	YU
contig02817	gi|67083885|gb|AAY66877.1| nonmuscle myosin essential light chai... 87	3E-16	Z
contig04801	gi|156555332|ref|XP_001604103.1| PREDICTED: similar to parvin [N... 87	3E-16	Z
contig07723	gi|194670334|ref|XP_612559.2| PREDICTED: acidic (leucine-rich) n... 88	2E-16	DR
contig02622	gi|189540623|ref|XP_684202.3| PREDICTED: similar to Ubiquitin sp... 88	2E-16	O
contig08640	gi|126304111|ref|XP_001381909.1| PREDICTED: similar to FrzA [Mon... 88	2E-16	T
contig02331	gi|149380781|gb|ABR24785.1| glutathione S-transferase mu class [... 89	1E-16	O
contig03943	gi|6318665|gb|AAF06994.1|AF167366_1 T-complex protein 1 gamma su... 89	1E-16	O
contig10839	gi|195479503|ref|XP_002100910.1| GE15910 [*Drosophila yakuba*] >gn... 89	1E-16	O
contig10311	gi|158299486|ref|XP_319605.4| AGAP008863-PA [*Anopheles gambiae* s... 89	1E-16	T
contig03892	gi|156402263|ref|XP_001639510.1| predicted protein [*Nematostella*... 89	1E-16	U
contig10172	gi|156554310|ref|XP_001602669.1| PREDICTED: similar to CG6775-PC... 89	1E-16	U
contig03228	gi|195457232|ref|XP_002075484.1| GK18337 [*Drosophila willistoni*]... 89	8E-17	O
contig04998	gi|142976617|ref|NP_001073358.2| selenophosphate synthetase 2 [R... 89	8E-17	T
contig07532	gi|156554719|ref|XP_001605102.1| PREDICTED: similar to conserved... 90	7E-17	T
contig00107	gi|114842185|dbj|BAF32577.1| cytoplasmic actin [*Varroa destructor*] 90	6E-17	Z
contig01051	gi|94734234|emb|CAK11350.1| novel protein (zgc:66267) [*Danio rerio*] 91	4E-17	O
contig12479	gi|157745561|ref|XP_001667877.1| Hypothetical protein CBG14979 [... 94	4E-17	O
contig03120	gi|34539117|gb|AAQ74442.1| glutathione S-transferase [*Rhipicepha*... 91	3E-17	O
contig07628	gi|195457050|ref|XP_002075403.1| GK17732 [*Drosophila willistoni*]... 91	3E-17	T
contig08978	gi|156554755|ref|XP_001605584.1| PREDICTED: similar to ras-relat... 91	3E-17	TU
contig09646	gi|189241915|ref|XP_971248.2| PREDICTED: similar to Putative GTP... 91	3E-17	TU
contig11500	gi|54400522|ref|NP_001006010.1| hypothetical protein LOC449989 [... 91	2E-17	DV
contig09144	gi|158297247|ref|XP_317515.4| AGAP007957-PA [*Anopheles gambiae* s... 92	2E-17	M
contig01114	gi|194853503|ref|XP_001968175.1| GG24722 [*Drosophila erecta*] >gn... 92	2E-17	O
contig09962	gi|71659830|ref|XP_821635.1| proteasome beta 2 subunit, putative... 88	2E-17	O
contig00898	gi|158289971|ref|XP_311569.4| AGAP010376-PA [*Anopheles gambiae* s... 92	2E-17	T
contig01553	gi|121309135|dbj|BAF43802.1| galectin [*Ornithodoros moubata*] >gn... 92	2E-17	W
contig09402	gi|195454473|ref|XP_002074253.1| GK18416 [*Drosophila willistoni*]... 92	2E-17	YU
contig06609	gi|156057557|ref|XP_001594702.1| conserved hypothetical protein ... 92	2E-17	Z
contig09779	gi|109104620|ref|XP_001110230.1| PREDICTED: similar to Tubulin a... 92	2E-17	Z
contig04686	gi|55926117|ref|NP_001007498.1| bub3 protein [*Xenopus tropicalis*... 92	1E-17	D
contig06446	gi|194764889|ref|XP_001964560.1| GF22987 [*Drosophila ananassae*] ... 92	1E-17	O
contig01892	gi|156555566|ref|XP_001604721.1| PREDICTED: similar to calcineur... 92	1E-17	T
contig02566	gi|193669177|ref|XP_001945333.1| PREDICTED: similar to AGAP00302... 92	1E-17	U
contig07422	gi|194213458|ref|XP_001496149.2| PREDICTED: similar to RAB6A, me... 92	1E-17	U
contig08607	gi|91079294|ref|XP_966628.1| PREDICTED: similar to CG8707 CG8707... 92	1E-17	U
contig11289	gi|195429527|ref|XP_002062810.1| GK19497 [*Drosophila willistoni*]... 93	1E-17	U
contig00318	gi|114052272|ref|NP_001040465.1| tropomyosin isoform 2 [*Bombyx m*... 92	1E-17	Z
contig04037	gi|156394055|ref|XP_001636642.1| predicted protein [*Nematostella*... 93	8E-18	O
contig05755	gi|91093675|ref|XP_969585.1| PREDICTED: similar to AGAP004340-PA... 93	8E-18	T
contig09284	gi|50749392|ref|XP_421617.1| PREDICTED: hypothetical protein [*Ga*... 93	8E-18	U

Contig	BLAST result	e value	Class
contig05883	gi|195964871|gb|ACG60423.1| heat shock protein 70-2 [*Tetranychus*... 60	7E-18	O
contig03321	gi|91088981|ref|XP_966828.1| PREDICTED: similar to CG4165 CG4165... 93	6E-18	O
contig07206	gi|47218760|emb|CAG02746.1| unnamed protein product [*Tetraodon n*... 93	6E-18	O
contig08582	gi|189234451|ref|XP_967488.2| PREDICTED: similar to blue cheese ... 93	6E-18	TU
contig06440	gi|156537015|ref|XP_001608302.1| PREDICTED: similar to chaperoni... 94	3E-18	O
contig09565	gi|156555726|ref|XP_001601862.1| PREDICTED: similar to paraplegi... 94	3E-18	O
contig09743	gi|195457695|ref|XP_002075674.1| GK23547 [*Drosophila willistoni*]... 94	3E-18	O
contig05090	gi|118091839|ref|XP_421224.2| PREDICTED: similar to vesicle tran... 94	3E-18	U
contig02798	gi|170585966|ref|XP_001897752.1| Ubiquitin-conjugating enzyme E2... 95	2E-18	O
contig05521	gi|189525365|ref|XP_001341326.2| PREDICTED: similar to ubiquitin... 95	2E-18	O
contig11390	gi|118793755|ref|XP_321060.3| AGAP001999-PA [*Anopheles gambiae* s... 95	2E-18	T
contig02139	gi|124487934|gb|ABN12050.1| putative ribophorin II [*Maconellicoc*... 96	1E-18	O
contig07256	gi|183979376|dbj|BAG30740.1| muscle myosin heavy chain [*Papilio* ... 96	1E-18	Z
contig07267	gi|2947316|gb|AAC05388.1| fibulin-1, isoform C precursor [*Gallus*... 96	9E-19	T
contig09580	gi|193685937|ref|XP_001952603.1| PREDICTED: similar to sarcalume... 96	9E-19	TU
contig06290	gi|66565839|ref|XP_395065.2| PREDICTED: similar to Clathrin ligh... 96	9E-19	U
contig04475	gi|33598988|gb|AAP51387.1| constitutive heat shock protein HSC70... 64	8E-19	O
contig02453	gi|58390289|ref|XP_317621.2| AGAP007871-PA [*Anopheles gambiae* st... 98	6E-19	U
contig10970	gi|156543740|ref|XP_001605962.1| PREDICTED: similar to tripeptid... 97	5E-19	O
contig05415	gi|3913717|sp|O16118|GNAS_HOMAM Guanine nucleotide-binding prote... 97	5E-19	T
contig05989	gi|193624646|ref|XP_001952092.1| PREDICTED: similar to muscle my... 95	5E-19	Z
contig01112	gi|170581201|ref|XP_001895580.1| ubiquitin C-terminal hydrolase ... 97	3E-19	O
contig04551	gi|149412083|ref|XP_001510215.1| PREDICTED: hypothetical protein... 98	2E-19	T
contig08850	gi|72017227|ref|XP_791019.1| PREDICTED: similar to MGC82152 prot... 98	2E-19	T
contig09376	gi|168278389|dbj|BAG11074.1| coatomer subunit alpha [synthetic c... 98	2E-19	U
contig13287	gi|126323523|ref|XP_001364576.1| PREDICTED: hypothetical protein... 98	2E-19	U
contig01433	gi|156553669|ref|XP_001602360.1| PREDICTED: similar to beta-tubu... 98	2E-19	Z
contig08500	gi|148235319|ref|NP_001080290.1| ubiquitin-conjugating enzyme E2... 73	1E-19	O
contig10643	gi|147905141|ref|NP_001086744.1| G protein-coupled receptor 89 [... 99	1E-19	T
contig11655	gi|196000721|ref|XP_002110228.1| expressed hypothetical protein ... 99	1E-19	T
contig04567	gi|193587329|ref|XP_001949579.1| PREDICTED: similar to CG14232 C... 99	1E-19	U
contig00217	gi|195435101|ref|XP_002065540.1| GK14617 [*Drosophila willistoni*]... 99	8E-20	O
contig07687	gi|109079105|ref|XP_001094909.1| PREDICTED: ring finger protein ... 99	8E-20	O
contig02840	gi|195384084|ref|XP_002050748.1| GJ20037 [*Drosophila virilis*] >g... 100	7E-20	T
contig03407	gi|114637619|ref|XP_508413.2| PREDICTED: proteasome 26S ATPase s... 90	6E-20	O
contig06623	gi|110751295|ref|XP_623197.2| PREDICTED: similar to GDP dissocia... 100	6E-20	O
contig08754	gi|183979249|dbj|BAG30786.1| similar to CG2708-PA [*Papilio xuthus*] 100	6E-20	ODR
contig03192	gi|148231189|ref|NP_001085042.1| hypothetical protein LOC432109 ... 100	6E-20	Z
contig07454	gi|195553249|ref|XP_002076630.1| GD15163 [*Drosophila simulans*] >... 100	5E-20	T
contig04869	gi|198138224|gb|EDY70016.1| GA25794 [*Drosophila pseudoobscura* ps... 100	4E-20	Z
contig11791	gi|193783623|dbj|BAG53534.1| unnamed protein product [*Homo sapiens*] 100	3E-20	O
contig12186	gi|195150723|ref|XP_002016300.1| GL10567 [*Drosophila persimilis*]... 61	3E-20	T
contig05295	gi|170067248|ref|XP_001868406.1| translocon-associated protein s... 88	3E-20	U
contig00543	gi|170596615|ref|XP_001902831.1| FKBP-type peptidyl-prolyl cis-t... 103	2E-20	O
contig06494	gi|196000965|ref|XP_002110350.1| hypothetical protein TRIADDRAFT... 101	2E-20	U
contig02766	gi|170029190|ref|XP_001842476.1| myosin heavy chain [*Culex quinq*... 101	2E-20	Z
contig10612	gi|156538817|ref|XP_001607965.1| PREDICTED: similar to ubiquitin... 102	1E-20	O
contig01945	gi|113204600|gb|ABI34002.1| high-affinity serotonin transporter ... 102	1E-20	T
contig00795	gi|198138224|gb|EDY70016.1| GA25794 [*Drosophila pseudoobscura* ps... 102	1E-20	Z
contig06823	gi|158298304|ref|XP_554420.3| AGAP004023-PA [*Anopheles gambiae* s... 102	1E-20	ZU
contig03681	gi|195999642|ref|XP_002109689.1| hypothetical protein TRIADDRAFT... 102	7E-21	DTZ

Contig	BLAST result	e value	Class
contig02992	gi|91092996|ref|XP_968315.1| PREDICTED: similar to Cysteine-rich... 102	7E-21	Z
contig08545	gi|107910630|gb|ABF85673.1| 70 kD heat shock protein [*Rimicaris* ... 73	4E-21	O
contig04194	gi|37925239|gb|AAP59794.1| slow muscle myosin S1 heavy chain [*Ho*... 73	4E-21	Z
contig08013	gi|71896679|ref|NP_001026142.1| phosphatidylinositol-4-phosphate... 104	3E-21	T
contig01256	gi|114153122|gb|ABI52727.1| ADP ribosylation factor 79F [*Argas m*... 64	3E-21	U
contig02164	gi|115643098|ref|XP_001191223.1| PREDICTED: similar to suppresso... 103	3E-21	U
contig13056	gi|37903435|gb|AAO38846.1| actin [Danio rerio] >gnl|BL_ORD_ID|29... 104	3E-21	Z
contig13123	gi|37903435|gb|AAO38846.1| actin [Danio rerio] >gnl|BL_ORD_ID|29... 104	3E-21	Z
contig03884	gi|156553628|ref|XP_001600123.1| PREDICTED: hypothetical protein... 104	2E-21	O
contig11432	gi|66566513|ref|XP_395605.2| PREDICTED: similar to CG7830-PA [Ap... 104	2E-21	O
contig01265	gi|195020142|ref|XP_001985130.1| GH16896 [*Drosophila grimshawi*] ... 104	2E-21	Z
contig00991	gi|198137562|gb|EDY69675.1| GA25293 [*Drosophila pseudoobscura* ps... 105	1E-21	O
contig11560	gi|158288915|ref|XP_310731.4| AGAP000378-PA [*Anopheles gambiae* s... 107	1E-21	T
contig12416	gi|67083891|gb|AAY66880.1| D-dopachrome tautomerase [*Ixodes scap*... 107	1E-21	V
contig05448	gi|110759783|ref|XP_395212.3| PREDICTED: similar to Spectrin bet... 105	1E-21	Z
contig02282	gi|47229652|emb|CAG06848.1| unnamed protein product [*Tetraodon n*... 105	9E-22	O
contig12224	gi|115655582|ref|XP_780679.2| PREDICTED: similar to granulin [*St*... 106	9E-22	T
contig03250	gi|91082327|ref|XP_974606.1| PREDICTED: similar to prohibitin pr... 106	7E-22	O
contig02525	gi|26891614|gb|AAN78379.1| CG10174 protein [*Drosophila melanogas*... 106	7E-22	U
contig04765	gi|82621739|gb|ABB86551.1| proteosome PSMB6/9 protein [*Branchios*... 106	5E-22	O
contig05490	gi|91077138|ref|XP_971446.1| PREDICTED: similar to DnaJ homolog ... 107	4E-22	O
contig05522	gi|91075936|ref|XP_967560.1| PREDICTED: similar to AGAP002481-PA... 107	4E-22	O
contig08749	gi|49474021|ref|YP_032063.1| protease II [Bartonella quintana st... 97	4E-22	O
contig08798	gi|193617698|ref|XP_001948523.1| PREDICTED: similar to GA18789-P... 107	4E-22	O
contig09165	gi|66533395|ref|XP_393300.2| PREDICTED: similar to CG7033-PA, is... 68	4E-22	O
contig07757	gi|189525403|ref|XP_001921194.1| PREDICTED: similar to inositol ... 107	4E-22	T
contig06227	gi|4322296|gb|AAD16002.1| cAMP-dependent protein kinase catalyti... 107	3E-22	T
contig02836	gi|157111247|ref|XP_001651451.1| BRI1-KD interacting protein, pu... 107	3E-22	U
contig08586	gi|66553034|ref|XP_396707.2| PREDICTED: similar to Serine/threon... 108	2E-22	D
contig03707	gi|195457320|ref|XP_002075523.1| GK18527 [*Drosophila willistoni*]... 110	2E-22	O
contig05250	gi|193594276|ref|XP_001945478.1| PREDICTED: similar to proteasom... 108	2E-22	O
contig08559	gi|72012370|ref|XP_781234.1| PREDICTED: similar to p70 ribosomal... 108	2E-22	RT
contig01750	gi|123469147|ref|XP_001317787.1| AGC family protein kinase [*Tric*... 108	1E-22	D
contig05305	gi|146284648|ref|YP_001165601.1| cobyrinic acid a,c-diamide synt... 108	1E-22	D
contig00365	gi|195394999|ref|XP_002056124.1| GJ10398 [*Drosophila virilis*] >g... 108	1E-22	O
contig06195	gi|7416829|dbj|BAA94076.1| ubiquitin-activating enzyme E1 [*Caras*... 108	1E-22	O
contig10729	gi|156377225|ref|XP_001630757.1| predicted protein [*Nematostella*... 108	1E-22	O
contig03356	gi|58382750|ref|XP_312147.2| AGAP002777-PA [*Anopheles gambiae* st... 108	1E-22	T
contig05211	gi|156545774|ref|XP_001605636.1| PREDICTED: similar to Sec24B pr... 109	8E-23	U
contig12767	gi|160420131|ref|NP_001080570.1| dual specificity phosphatase 1 ... 109	8E-23	V
contig01933	gi|156375039|ref|XP_001629890.1| predicted protein [*Nematostella*... 110	6E-23	D
contig02789	gi|170043539|ref|XP_001849441.1| grpE [*Culex quinquefasciatus*] >... 109	6E-23	O
contig03560	gi|116488307|gb|ABJ98722.1| heat shock protein 71 [*Perna viridis*... 106	6E-23	O
contig04358	gi|139000435|dbj|BAF51711.1| tick legumain [*Haemaphysalis longic*... 109	6E-23	O
contig12259	gi|91082187|ref|XP_971460.1| PREDICTED: similar to conserved hyp... 113	6E-23	O
contig03280	gi|46048731|ref|NP_990565.1| hippocalcin-like 1 [*Gallus gallus*] ... 95	6E-23	T
contig04005	gi|62554177|dbj|BAD95607.1| WNT8C [*Gallus gallus*] 109	6E-23	T
contig07142	gi|193627205|ref|XP_001952587.1| PREDICTED: similar to AGAP00926... 109	6E-23	T
contig02883	gi|119352599|gb|ABL63902.1| capping protein beta subunit [*Xenopu*... 109	6E-23	Z
contig10158	gi|72064509|ref|XP_780270.1| PREDICTED: similar to chaperonin is... 110	4E-23	O
contig06098	gi|156380509|ref|XP_001631811.1| predicted protein [*Nematostella*... 110	4E-23	U

Contig	BLAST result	e value	Class
contig08540	gi|195450889|ref|XP_002072676.1| GK13729 [*Drosophila willistoni*]... 110	3E-23	Z
contig02122	gi|157132324|ref|XP_001655999.1| cathepsin l [*Aedes aegypti*] >gn... 111	2E-23	O
contig04751	gi|91091652|ref|XP_971071.1| PREDICTED: similar to mitochondrial... 111	2E-23	O
contig11044	gi|66813248|ref|XP_640803.1| hypothetical protein DDBDRAFT_02039... 104	2E-23	O
contig04083	gi|126324876|ref|XP_001379073.1| PREDICTED: similar to clathrin ... 111	2E-23	U
contig05088	gi|189526748|ref|XP_694714.3| PREDICTED: similar to Golgi-specif... 111	2E-23	U
contig05429	gi|156542546|ref|XP_001599253.1| PREDICTED: similar to probable ... 111	2E-23	UO
contig10851	gi|19074903|ref|NP_586409.1| PROTEASOME REGULATORY SUBUNIT YTA6 ... 112	1E-23	O
contig11670	gi|91093707|ref|XP_967114.1| PREDICTED: similar to sex-determini... 112	1E-23	OT
contig06681	gi|189537228|ref|XP_001921364.1| PREDICTED: similar to AP-3 comp... 112	1E-23	U
contig02564	gi|154814668|gb|ABS87368.1| lospin 16 [*Amblyomma americanum*] 71	1E-23	V
contig07322	gi|91076138|ref|XP_970159.1| PREDICTED: similar to actin binding... 112	1E-23	W
contig10020	gi|156368061|ref|XP_001627515.1| predicted protein [*Nematostella*... 112	9E-24	O
contig01025	gi|193657081|ref|XP_001945441.1| PREDICTED: similar to ubiquitin... 112	8E-24	O
contig02263	gi|51011554|gb|AAT92186.1| heat shock protein 10 [*Ixodes pacificus*] 114	7E-24	O
contig12574	gi|109104965|ref|XP_001091374.1| PREDICTED: cathepsin D isoform ... 112	7E-24	O
contig07517	gi|189234922|ref|XP_970841.2| PREDICTED: similar to myosin light... 112	7E-24	Z
contig09419	gi|149633775|ref|XP_001513271.1| PREDICTED: similar to ubiquitin... 113	5E-24	O
contig09046	gi|7330345|gb|AAB24072.2| G-protein alpha i subunit [*Homarus ame*... 113	4E-24	DT
contig10352	gi|110771394|ref|XP_001123288.1| PREDICTED: similar to Insulin-d... 113	4E-24	O
contig01567	gi|148558491|ref|YP_001257246.1| chaperonin GroEL [*Brucella ovis*... 114	3E-24	O
contig06860	gi|165905471|dbj|BAF99000.1| atypical protein kinase C [*Hemicent*... 110	3E-24	T
contig00572	gi|91081923|ref|XP_970724.1| PREDICTED: similar to DnaJ (Hsp40) ... 114	2E-24	O
contig00664	gi|58377313|ref|XP_309539.2| glutaredoxin (AGAP011107-PA) [*Anoph*... 114	2E-24	O
contig07580	gi|194758635|ref|XP_001961567.1| GF14871 [*Drosophila ananassae*] ... 114	2E-24	T
contig08536	gi|156549535|ref|XP_001602657.1| PREDICTED: similar to GA19430-P... 114	2E-24	T
contig13087	gi|42476045|ref|NP_599161.2| cathepsin D [*Rattus norvegicus*] >gn... 115	1E-24	O
contig07958	gi|193629709|ref|XP_001948291.1| PREDICTED: hypothetical protein... 115	1E-24	T
contig05337	gi|91081955|ref|XP_967420.1| PREDICTED: similar to cullin [*Tribo*... 115	8E-25	D
contig01368	gi|196004168|ref|XP_002111951.1| hypothetical protein TRIADDRAFT... 116	8E-25	Z
contig08436	gi|91077186|ref|XP_972614.1| PREDICTED: similar to cyclin-K [*Tri*... 116	7E-25	D
contig05122	gi|47216266|emb|CAG05962.1| unnamed protein product [*Tetraodon n*... 73	7E-25	O
contig09333	gi|65332126|gb|AAY42205.1| troponin T [*Haemaphysalis qinghaiensis*] 101	7E-25	Z
contig10687	gi|193624646|ref|XP_001952092.1| PREDICTED: similar to muscle my... 116	7E-25	Z
contig06781	gi|156374072|ref|XP_001629633.1| predicted protein [*Nematostella*... 116	6E-25	D
contig07660	gi|156352097|ref|XP_001622605.1| predicted protein [*Nematostella*... 116	6E-25	O
contig03145	gi|56412194|gb|AAV88596.1| Sparc [*Schistocerca gregaria*] 116	6E-25	W
contig11037	gi|71834414|ref|NP_001025299.1| dynamin 2 [*Danio rerio*] >gnl|BL_... 116	5E-25	UR
contig05915	gi|189234352|ref|XP_973750.2| PREDICTED: similar to spectrin [*Tr*... 116	5E-25	Z
contig12620	gi|170063951|ref|XP_001867326.1| lysosomal aspartic protease [*Cu*... 117	4E-25	O
contig10034	gi|29134779|dbj|BAC66140.1| projectin [*Procambarus clarkii*] 117	4E-25	Z
contig10243	gi|115654526|ref|XP_001176817.1| PREDICTED: similar to Structura... 117	3E-25	D
contig01287	gi|158298637|ref|XP_318831.4| AGAP009747-PA [*Anopheles gambiae* s... 119	2E-25	O
contig11938	gi|161788938|dbj|BAF95090.1| 2nd tick legumain [*Haemaphysalis lo*... 77	2E-25	O
contig04423	gi|189239939|ref|XP_001813596.1| PREDICTED: similar to Myosin he... 117	2E-25	Z
contig01212	gi|75029909|sp|Q4PMC9|PNO1_IXOSC RNA-binding protein pno1 >gnl|B... 118	1E-25	O
contig08163	gi|112983414|ref|NP_001036985.1| heat shock protein hsp21.4 [Bom... 118	1E-25	O
contig10087	gi|195349292|ref|XP_002041179.1| GM15181 [*Drosophila sechellia*] ... 119	1E-25	O
contig02167	gi|73966641|ref|XP_867227.1| PREDICTED: similar to Clathrin heav... 71	1E-25	U
contig09818	gi|195129812|ref|XP_002009348.1| GI15281 [*Drosophila mojavensis*]... 118	1E-25	Z
contig03518	gi|110762560|ref|XP_391992.3| PREDICTED: similar to unconvention... 119	8E-26	N

Contig	BLAST result	e value	Class
contig10404	gi|156537047|ref|XP_001601463.1| PREDICTED: similar to GA15568-P... 119	6E-26	DO
contig04255	gi|114051682|ref|NP_001040173.1| calcium-binding protein p22 [*Bo*... 119	6E-26	T
contig11382	gi|66501010|ref|XP_392587.2| PREDICTED: similar to alien CG9556-... 120	4E-26	OT
contig01177	gi|148608643|gb|ABQ95654.1| mitogen-activated protein kinase kin... 120	3E-26	T
contig05765	gi|47215026|emb|CAG01850.1| unnamed protein product [*Tetraodon n*... 120	3E-26	T
contig00126	gi|157123632|ref|XP_001660237.1| cyclin B3 [*Aedes aegypti*] >gnl|... 121	2E-26	D
contig02950	gi|110665909|gb|ABG81495.1| ubiquitin [*Mayetiola destructor*] 121	2E-26	O
contig05161	gi|126327605|ref|XP_001376365.1| PREDICTED: hypothetical protein... 122	2E-26	O
contig01116	gi|66500538|ref|XP_392119.2| PREDICTED: similar to Phosphatidyli... 122	2E-26	T
contig06056	gi|121543726|gb|ABM55548.1| putative peptidyl-prolyl cis-trans i... 122	9E-27	O
contig01608	gi|91078858|ref|XP_972061.1| PREDICTED: similar to thymus-specif... 122	9E-27	OR
contig04919	gi|170049784|ref|XP_001870925.1| mitochondrial import inner memb... 122	7E-27	U
contig06806	gi|37651156|dbj|BAC98949.1| beta-tubulin [*Octopus vulgaris*] 122	7E-27	Z
contig06184	gi|110761364|ref|XP_001121589.1| PREDICTED: similar to Alpha-act... 106	6E-27	Z
contig00156	gi|118104159|ref|XP_425038.2| PREDICTED: similar to cathepsin L ... 96	5E-27	O
contig12240	gi|33348836|gb|AAQ16118.1| cathepsin L-like cysteine proteinase ... 123	5E-27	O
contig03683	gi|158287647|ref|XP_309631.4| AGAP004079-PA [*Anopheles gambiae* s... 123	5E-27	T
contig01298	gi|156549140|ref|XP_001607681.1| PREDICTED: similar to microtubu... 123	5E-27	TR
contig11663	gi|109127465|ref|XP_001094589.1| PREDICTED: TNF receptor-associa... 124	3E-27	O
contig03395	gi|195453988|ref|XP_002074034.1| GK12822 [*Drosophila willistoni*]... 124	2E-27	M
contig08973	gi|195111606|ref|XP_002000369.1| GI22559 [*Drosophila mojavensis*]... 124	2E-27	O
contig04961	gi|110763838|ref|XP_395927.3| PREDICTED: similar to Lk6 CG17342-... 124	2E-27	T
contig11832	gi|189238236|ref|XP_972123.2| PREDICTED: similar to AGAP009953-P... 124	2E-27	T
contig04384	gi|193620299|ref|XP_001944741.1| PREDICTED: similar to ring fing... 125	1E-27	O
contig06643	gi|170045205|ref|XP_001850207.1| past-1 [*Culex quinquefasciatus*]... 125	1E-27	TU
contig07977	gi|148228813|ref|NP_001086997.1| N-ethylmaleimide-sensitive fact... 125	1E-27	U
contig03684	gi|193683746|ref|XP_001950805.1| PREDICTED: similar to alpha act... 125	1E-27	Z
contig02722	gi|159112802|ref|XP_001706629.1| Peptidyl-prolyl cis-trans isome... 104	9E-28	O
contig10117	gi|125980157|ref|XP_001354111.1| GA19652-PA [*Drosophila pseudoob*... 125	8E-28	O
contig04642	gi|110761559|ref|XP_395279.3| PREDICTED: similar to Darkener of ... 125	8E-28	T
contig04253	gi|156542889|ref|XP_001600957.1| PREDICTED: similar to stromal a... 126	6E-28	D
contig05790	gi|193690576|ref|XP_001944217.1| PREDICTED: similar to CG34352 C... 126	6E-28	T
contig03791	gi|156541797|ref|XP_001602382.1| PREDICTED: similar to SD01613p ... 126	5E-28	O
contig11656	gi|47550797|ref|NP_999924.1| legumain [Danio rerio] >gnl|BL_ORD_... 126	5E-28	O
contig06758	gi|189239939|ref|XP_001813596.1| PREDICTED: similar to Myosin he... 79	4E-28	Z
contig00048	gi|193599208|ref|XP_001947519.1| PREDICTED: similar to meningiom... 128	3E-28	O
contig00342	gi|193643700|ref|XP_001950340.1| PREDICTED: similar to homologue... 121	3E-28	O
contig03374	gi|195996309|ref|XP_002108023.1| hypothetical protein TRIADDRAFT... 128	2E-28	M
contig00202	gi|195399558|ref|XP_002058386.1| GJ14386 [*Drosophila virilis*] >g... 129	2E-28	O
contig08261	gi|118092150|ref|XP_421350.2| PREDICTED: hypothetical protein [*G*... 128	2E-28	U
contig03612	gi|157168009|ref|XP_001663284.1| adenylyl cyclase-associated pro... 128	2E-28	ZT
contig02349	gi|124487748|gb|ABN11961.1| putative T-complex protein 1 subunit... 129	1E-28	O
contig03276	gi|118593492|ref|ZP_01550872.1| Esterase/lipase/thioesterase, ac... 128	1E-28	O
contig03868	gi|194043678|ref|XP_001925937.1| PREDICTED: proteasome (prosome,... 128	1E-28	O
contig00355	gi|145499164|ref|XP_001435568.1| hypothetical protein GSPATT0003... 130	9E-29	RT
contig08385	gi|170579335|ref|XP_001894786.1| DnaJ homolog subfamily B member... 129	7E-29	O
contig03904	gi|195146696|ref|XP_002014320.1| GL19137 [*Drosophila persimilis*]... 99	5E-29	YU
contig00617	gi|149428150|ref|XP_001511081.1| PREDICTED: similar to p85 beta ... 130	4E-29	T
contig04880	gi|195572192|ref|XP_002104080.1| GD20772 [*Drosophila simulans*] >... 130	4E-29	U
contig00276	gi|195029561|ref|XP_001987640.1| GH19845 [*Drosophila grimshawi*] ... 131	3E-29	O
contig04264	gi|91080285|ref|XP_973904.1| PREDICTED: similar to B-box type zi... 130	3E-29	O

Contig	BLAST result	e value	Class
contig06586	gi|112983414|ref|NP_001036985.1| heat shock protein hsp21.4 [*Bom*... 130	3E-29	O
contig11024	gi|309462|gb|AAA39948.1| protein kinase 132	2E-29	D
contig06040	gi|126333982|ref|XP_001364360.1| PREDICTED: similar to DnaJ-like... 131	2E-29	O
contig07051	gi|193699959|ref|XP_001951177.1| PREDICTED: similar to cytochrom... 131	2E-29	O
contig09684	gi|193621494|ref|XP_001952471.1| PREDICTED: similar to oligosacc... 131	2E-29	O
contig12697	gi|139439837|ref|ZP_01773214.1| Hypothetical protein COLAER_0224... 79	2E-29	T
contig03373	gi|189234352|ref|XP_973750.2| PREDICTED: similar to spectrin [*Tr*... 130	2E-29	Z
contig05234	gi|126293910|ref|XP_001363445.1| PREDICTED: similar to ubiquitin... 133	1E-29	O
contig10060	gi|91080285|ref|XP_973904.1| PREDICTED: similar to B-box type zi... 132	1E-29	O
contig12120	gi|189517055|ref|XP_001341446.2| PREDICTED: similar to heat shoc... 132	1E-29	O
contig01525	gi|170595779|ref|XP_001902516.1| Transmembrane protein [*Brugia m*... 132	1E-29	U
contig12011	gi|195381281|ref|XP_002049382.1| GJ20779 [*Drosophila virilis*] >g... 133	5E-30	T
contig08026	gi|156454274|gb|ABU63810.1| heat shock protein 70 [*Hesiolyra bergi*] 128	4E-30	O
contig00031	gi|161661029|gb|ABX75381.1| cysteine and glycine-rich protein [*L*... 136	4E-30	TZ
contig03985	gi|47086637|ref|NP_997868.1| hypoxia up-regulated 1 [*Danio rerio*... 134	2E-30	O
contig06312	gi|67083917|gb|AAY66893.1| SCF ubiquitin ligase complex [*Ixodes* ... 135	1E-30	O
contig07304	gi|158293554|ref|XP_314892.4| AGAP008768-PA [*Anopheles gambiae* s... 135	1E-30	O
contig12285	gi|72062512|ref|XP_796204.1| PREDICTED: similar to ENSANGP000000... 135	1E-30	O
contig01521	gi|193683394|ref|XP_001944981.1| PREDICTED: similar to AGAP00627... 135	1E-30	T
contig02095	gi|156550987|ref|XP_001604363.1| PREDICTED: similar to endomembr... 135	1E-30	U
contig06031	gi|91087767|ref|XP_975031.1| PREDICTED: similar to Vacuolar prot... 73	1E-30	U
contig06256	gi|170032989|ref|XP_001844362.1| adiponectin receptor protein 2 ... 135	8E-31	RT
contig05963	gi|146189553|emb|CAM91778.1| hypothetical protein [*Platynereis d*... 137	2E-31	Z
contig01638	gi|126336219|ref|XP_001366393.1| PREDICTED: similar to nuclear r... 83	1E-31	T
contig04666	gi|110756153|ref|XP_001120355.1| PREDICTED: similar to Arpc3B CG... 131	1E-31	Z
contig06110	gi|156374137|ref|XP_001629665.1| predicted protein [*Nematostella*... 140	7E-32	U
contig03367	gi|161661029|gb|ABX75381.1| cysteine and glycine-rich protein [*L*... 142	5E-32	TZ
contig05354	gi|115655402|ref|XP_796587.2| PREDICTED: hypothetical protein [*S*... 140	4E-32	U
contig10346	gi|91087807|ref|XP_967674.1| PREDICTED: similar to vacuolar prot... 140	4E-32	U
contig11738	gi|189236765|ref|XP_975383.2| PREDICTED: similar to CG14407 CG14... 142	3E-32	O
contig06424	gi|82906177|ref|XP_890535.1| PREDICTED: similar to ubiquitin-con... 140	2E-32	O
contig00093	gi|158294130|ref|XP_315412.4| AGAP005402-PA [*Anopheles gambiae* s... 142	1E-32	RD
contig00799	gi|115712025|ref|XP_787000.2| PREDICTED: similar to apoptosis-li... 142	1E-32	T
contig01126	gi|157118476|ref|XP_001659125.1| calponin/transgelin [*Aedes aegy*... 144	1E-32	Z
contig02679	gi|149409055|ref|XP_001508583.1| PREDICTED: similar to Sp6 trans... 142	8E-33	O
contig09223	gi|91086195|ref|XP_971475.1| PREDICTED: similar to protein trans... 142	6E-33	U
contig05265	gi|156399594|ref|XP_001638586.1| predicted protein [*Nematostella*... 143	5E-33	T
contig11090	gi|118789340|ref|XP_317352.3| AGAP008105-PA [*Anopheles gambiae* s... 143	4E-33	D
contig03663	gi|110751295|ref|XP_623197.2| PREDICTED: similar to GDP dissocia... 143	4E-33	O
contig00984	gi|126330898|ref|XP_001376794.1| PREDICTED: hypothetical protein... 144	4E-33	T
contig05075	gi|194219924|ref|XP_001917962.1| PREDICTED: similar to protein p... 143	4E-33	T
contig05930	gi|156544986|ref|XP_001607995.1| PREDICTED: similar to clathrin ... 143	4E-33	U
contig02834	gi|193714972|ref|XP_001951659.1| PREDICTED: similar to Ring fing... 144	3E-33	O
contig11795	gi|91077294|ref|XP_968496.1| PREDICTED: similar to thymosin beta... 146	2E-33	N
contig03132	gi|194766555|ref|XP_001965390.1| GF20636 [*Drosophila ananassae*] ... 144	2E-33	O
contig12035	gi|91082073|ref|XP_966419.1| PREDICTED: similar to 26S proteasom... 144	2E-33	O
contig05230	gi|195394485|ref|XP_002055873.1| GJ10528 [*Drosophila virilis*] >g... 144	2E-33	T
contig11223	gi|47216032|emb|CAG11363.1| unnamed protein product [*Tetraodon n*... 145	2E-33	T
contig07735	gi|161661029|gb|ABX75381.1| cysteine and glycine-rich protein [*L*... 145	2E-33	TZ
contig02930	gi|66546674|ref|XP_395448.2| PREDICTED: similar to Cbl CG7037-PB... 144	2E-33	V
contig12254	gi|196009742|ref|XP_002114736.1| hypothetical protein TRIADDRAFT... 147	1E-33	O

Contig	BLAST result	e value	Class
contig10153	gi|189236779|ref|XP_967166.2| PREDICTED: similar to reticulon/no... 146	1E-33	U
contig11103	gi|58389525|ref|XP_317088.2| thioester-containing protein (AGAP0... 147	8E-34	O
contig07176	gi|28422163|gb|AAH46839.1| Plk-prov protein [*Xenopus laevis*] 147	6E-34	D
contig00996	gi|24652326|ref|NP_724885.1| 14-3-3zeta CG17870-PA, isoform A [*D*... 146	6E-34	O
contig08628	gi|193678945|ref|XP_001949533.1| PREDICTED: similar to CG10542 C... 146	6E-34	O
contig05200	gi|189234077|ref|XP_001812066.1| PREDICTED: similar to fimbrin/p... 146	6E-34	Z
contig03698	gi|170040959|ref|XP_001848248.1| integrin beta-PS [*Culex quinque*... 92	5E-34	TW
contig08387	gi|195331895|ref|XP_002032634.1| GM20894 [Drosophila sechellia] ... 146	5E-34	U
contig06929	gi|156548783|ref|XP_001604963.1| PREDICTED: similar to vacuolar ... 146	4E-34	U
contig04517	gi|110759191|ref|XP_393334.3| PREDICTED: similar to Myosin heavy... 146	4E-34	Z
contig11697	gi|157114579|ref|XP_001652323.1| calponin/transgelin [*Aedes aegy*... 147	4E-34	Z
contig01474	gi|157112080|ref|XP_001651784.1| proteasome subunit alpha type [... 148	2E-34	O
contig03196	gi|50756599|ref|XP_415234.1| PREDICTED: similar to Kelch-like 22... 147	2E-34	TR
contig04944	gi|114153202|gb|ABI52767.1| serpin [*Argas monolakensis*] 148	1E-34	V
contig03375	gi|114153122|gb|ABI52727.1| ADP ribosylation factor 79F [*Argas m*... 149	9E-35	U
contig06587	gi|194388764|dbj|BAG60350.1| unnamed protein product [*Homo sap*iens] 149	7E-35	O
contig03215	gi|196476637|gb|ACG76186.1| thioredoxin peroxidase [*Amblyomma am*... 151	4E-35	O
contig06208	gi|189241320|ref|XP_966927.2| PREDICTED: similar to CG5871 CG587... 151	2E-35	O
contig03260	gi|195153445|ref|XP_002017636.1| GL17205 [*Drosophila persimilis*]... 153	2E-35	U
contig00571	gi|189531123|ref|XP_001920415.1| PREDICTED: similar to nuclear p... 150	2E-35	YU
contig00106	gi|157779728|gb|ABV71392.1| actin [*Haemaphysalis longicornis*] 151	2E-35	Z
contig00990	gi|24584702|ref|NP_724004.1| Myosin heavy chain CG17927-PD, isof... 89	2E-35	Z
contig12237	gi|167683074|gb|ABZ91679.1| actin [*Uloborus diversus*] 151	2E-35	Z
contig13014	gi|126291135|ref|XP_001371416.1| PREDICTED: hypothetical protein... 154	2E-35	Z
contig03988	gi|156390723|ref|XP_001635419.1| predicted protein [*Nematostella*... 152	1E-35	D
contig01246	gi|110756772|ref|XP_001121636.1| PREDICTED: similar to peanut CG... 152	1E-35	DZU
contig11905	gi|167387323|ref|XP_001738112.1| cysteine proteinase 3 precursor... 153	9E-36	O
contig01431	gi|156388328|ref|XP_001634653.1| predicted protein [*Nematostella*... 155	6E-36	O
contig09966	gi|66511475|ref|XP_394621.2| PREDICTED: similar to -Adaptin CG42... 152	6E-36	U
contig01872	gi|110762076|ref|XP_625049.2| PREDICTED: similar to CG2862-PA, i... 114	4E-36	T
contig04299	gi|157132436|ref|XP_001662562.1| cytochrome c oxidase assembly p... 154	2E-36	O
contig06481	gi|146285369|gb|ABQ18260.1| protein serine/threonine phosphatase... 154	2E-36	TR
contig03333	gi|156371405|ref|XP_001628754.1| predicted protein [*Nematostella*... 155	1E-36	O
contig07191	gi|125807245|ref|XP_001360320.1| GA13759-PA [*Drosophila pseudoob*... 157	4E-37	O
contig07219	gi|12060812|gb|AAG48248.1|AF282407_1 p70 ribosomal protein S6 ki... 144	3E-37	RT
contig06802	gi|58332574|ref|NP_001011361.1| CDC-like kinase 2 [*Xenopus tropi*... 158	1E-37	T
contig10329	gi|118781781|ref|XP_311856.3| AGAP003021-PA [*Anopheles gambiae* s... 158	1E-37	U
contig12027	gi|82698821|gb|ABB89211.1| troponin I protein [*Rhipicephalus hae*... 160	1E-37	Z
contig11605	gi|73975640|ref|XP_858570.1| PREDICTED: similar to calumenin iso... 162	5E-38	TU
contig04584	gi|19112602|ref|NP_595810.1| chaperonin-containing T-complex gam... 162	1E-38	O
contig03977	gi|194673970|ref|XP_001255255.2| PREDICTED: similar to MAP kinas... 162	1E-38	T
contig00030	gi|161661029|gb|ABX75381.1| cysteine and glycine-rich protein [*L*... 166	8E-39	TZ
contig11290	gi|62178034|gb|AAX73177.1| putative heat shock 70 kDa protein [*E*... 162	6E-39	O
contig03652	gi|147907369|ref|NP_001087937.1| mec-12 protein [*Xenopus laevis*]... 90	5E-39	Z
contig05077	gi|66523378|ref|XP_394044.2| PREDICTED: similar to Cullin-1 (CUL... 165	1E-39	D
contig10928	gi|91086477|ref|XP_975920.1| PREDICTED: similar to microtubule-a... 165	1E-39	DZ
contig11334	gi|156538136|ref|XP_001608256.1| PREDICTED: similar to proteasom... 166	1E-39	O
contig08487	gi|71897157|ref|NP_001026081.1| ubiquitination factor E4A [*Gallu*... 165	9E-40	O
contig05064	gi|66499186|ref|XP_395748.2| PREDICTED: similar to FK506-binding... 136	4E-40	O
contig05049	gi|125842482|ref|XP_690505.2| PREDICTED: wu:fc07b10 [*Danio rerio*] 167	3E-40	O
contig07609	gi|193676530|ref|XP_001945130.1| PREDICTED: similar to four and ... 167	2E-40	TZR

Contig	BLAST result	e value	Class
contig00861	gi|39725991|gb|AAR29947.1| calreticulin [Haemaphysalis leporispa... 170	1E-40	O
contig03762	gi|161669254|gb|ABX75479.1| myosin heavy chain [*Lycosa singorien*... 168	1E-40	Z
contig13074	gi|47230018|emb|CAG10432.1| unnamed protein product [*Tetraodon n*... 169	6E-41	O
contig05373	gi|189236792|ref|XP_966417.2| PREDICTED: similar to protein phos... 170	3E-41	TR
contig02093	gi|53828193|emb|CAH58743.1| putative glutahione S-transferase, d... 174	1E-41	O
contig02957	gi|156404512|ref|XP_001640451.1| predicted protein [*Nematostella*... 102	1E-41	U
contig08269	gi|91083895|ref|XP_974479.1| PREDICTED: similar to ATPase family... 172	6E-42	O
contig00103	gi|81096421|ref|ZP_00874766.1| hypothetical protein SsuiDRAFT_16... 104	6E-42	W
contig00913	gi|38147395|gb|AAR12010.1| cathepsin L-like proteinase [*Triatoma*... 117	3E-42	O
contig06687	gi|156398911|ref|XP_001638431.1| predicted protein [*Nematostella*... 174	3E-42	ZT
contig04180	gi|67083919|gb|AAY66894.1| defender against cell death 1 [*Ixodes*... 175	2E-42	DO
contig01697	gi|147901313|ref|NP_001080849.1| von Hippel-Lindau binding prote... 134	2E-42	O
contig11291	gi|147905905|ref|NP_001082504.1| similar to ribophorin I [*Xenopu*... 154	2E-42	O
contig11904	gi|33667952|gb|AAQ24553.1| Blo t profilin allergen [*Blomia tropi*... 178	1E-42	Z
contig02392	gi|197127389|gb|ACH43887.1| putative Proteasome subunit alpha ty... 176	5E-43	O
contig01633	gi|91077058|ref|XP_968505.1| PREDICTED: similar to karyopherin a... 176	5E-43	U
contig12506	gi|55168340|gb|AAV44205.1| unknow protein [*Oryza sativa* (*japonic*... 115	5E-43	W
contig01541	gi|168002792|ref|XP_001754097.1| predicted protein [*Physcomitrel*... 177	4E-43	O
contig13129	gi|118429511|gb|ABK91803.1| aspartic protease precursor [*Clonorc*... 177	3E-43	O
contig03055	gi|193702351|ref|XP_001949887.1| PREDICTED: similar to integrin ... 177	3E-43	TW
contig02580	gi|22901939|gb|AAN10129.1| alpha-2-macroglobulin precursor splic... 180	2E-43	O
contig13020	gi|158634610|gb|ABW76151.1| myosin II regulatory light chain [*Av*... 181	6E-44	Z
contig04294	gi|189241696|ref|XP_970104.2| PREDICTED: similar to LIM protein ... 136	5E-44	TZ
contig02421	gi|189238186|ref|XP_966734.2| PREDICTED: similar to GA21791-PA [... 134	5E-44	Z
contig05027	gi|158294889|ref|XP_315883.4| AGAP005857-PA [*Anopheles gambiae* s... 180	4E-44	Z
contig02509	gi|91077396|ref|XP_975299.1| PREDICTED: similar to chaperonin [*T*... 148	2E-44	O
contig02749	gi|194389846|dbj|BAG60439.1| unnamed protein product [*Homo sapiens*] 183	2E-44	U
contig03517	gi|170585438|ref|XP_001897491.1| Tubulin alpha chain [*Brugia mal*... 181	2E-44	Z
contig13173	gi|1389604|gb|AAC59905.1| acetylcholinesterase 186	9E-45	T
contig05955	gi|91081399|ref|XP_972623.1| PREDICTED: similar to cyclin a [*Tri*... 184	8E-45	D
contig01281	gi|195110563|ref|XP_001999849.1| GI22847 [*Drosophila mojavensis*]... 186	1E-45	U
contig00176	gi|67083759|gb|AAY66814.1| selenium dependent salivary glutathio... 188	6E-46	O
contig13272	gi|195472863|ref|XP_002088718.1| GE11255 [*Drosophila yakuba*] >gn... 190	5E-46	T
contig11526	gi|114051243|ref|NP_001040382.1| FK506-binding protein [*Bombyx m*... 189	2E-46	O
contig04115	gi|194889394|ref|XP_001977075.1| GG18832 [*Drosophila erecta*] >gn... 191	7E-47	O
contig02365	gi|195436762|ref|XP_002066324.1| GK18232 [*Drosophila willistoni*]... 189	6E-47	Z
contig03783	gi|193617734|ref|XP_001946433.1| PREDICTED: similar to zinc fing... 189	5E-47	O
contig12063	gi|118102310|ref|XP_419249.2| PREDICTED: hypothetical protein [G... 191	3E-47	Z
contig04858	gi|170038216|ref|XP_001846948.1| dolichyl-diphosphooligosacchari... 192	2E-47	O
contig11169	gi|41393177|ref|NP_958915.1| mitogen-activated protein kinase 3 ... 191	2E-47	T
contig12263	gi|156551872|ref|XP_001603255.1| PREDICTED: similar to GA12600-P... 193	2E-47	V
contig11552	gi|45822201|emb|CAE47497.1| cathepsin L-like proteinase [*Diabrot*... 151	1E-47	O
contig11512	gi|161671310|gb|ABX75507.1| cofilin [*Lycosa singoriensis*] 196	1E-47	Z
contig00949	gi|156547852|ref|XP_001605005.1| PREDICTED: similar to ENSANGP00... 195	7E-48	U
contig03140	gi|187177323|ref|NP_001119639.1| Sec61 alpha 1 subunit [*Acyrthos*... 194	6E-48	UO
contig01889	gi|114153020|gb|ABI52676.1| Ca2+ calmodulin dependent protein ki... 194	3E-48	T
contig07057	gi|58257644|dbj|BAA07894.2| KIAA0098 protein [*Homo sapiens*] 194	2E-48	O
contig00449	gi|147905680|ref|NP_001080243.1| proteasome (prosome, macropain)... 196	1E-48	O
contig00144	gi|197246922|gb|AAI69141.1| Aurora kinase A [*Xenopus tropicalis*] 197	8E-49	D
contig04218	gi|77166828|gb|ABA62390.1| phospholipid-hydroperoxide glutathion... 196	8E-49	O
contig13217	gi|41053411|ref|NP_956969.1| RER1 retention in endoplasmic retic... 197	5E-49	U

Contig	BLAST result	e value	Class
contig11136	gi|195036412|ref|XP_001989664.1| GH18918 [*Drosophila grimshawi*] ... 200	3E-49	U
contig01790	gi|195046036|ref|XP_001992076.1| GH24410 [*Drosophila grimshawi*] ... 197	3E-49	Z
contig00691	gi|156537928|ref|XP_001608152.1| PREDICTED: similar to ENSANGP00... 198	2E-49	O
contig12330	gi|94966875|ref|NP_001035631.1| ubiquitin carboxyl-terminal este... 199	2E-49	O
contig04016	gi|110768448|ref|XP_624843.2| PREDICTED: similar to cheerio CG39... 197	2E-49	Z
contig09395	gi|74190898|dbj|BAE28230.1| unnamed protein product [*Mus musculus*] 112	1E-49	U
contig01456	gi|196011966|ref|XP_002115846.1| phosphatase 2A [*Trichoplax adha*... 200	7E-50	T
contig01363	gi|18043133|gb|AAH19682.1| Ctsd protein [*Mus musculus*] 202	1E-50	O
contig03692	gi|91078920|ref|XP_973689.1| PREDICTED: similar to ubiquitin-con... 202	7E-51	O
contig11163	gi|72136076|ref|XP_781152.1| PREDICTED: similar to thioredoxin f... 203	4E-51	O
contig02719	gi|147907369|ref|NP_001087937.1| mec-12 protein [*Xenopus laevis*]... 205	1E-51	Z
contig01425	gi|66503873|ref|XP_624697.1| PREDICTED: similar to 26S proteasom... 205	8E-52	O
contig00340	gi|3435078|gb|AAD05373.1| cholinesterase 1 [*Branchiostoma floridae*] 209	8E-52	T
contig04978	gi|193676530|ref|XP_001945130.1| PREDICTED: similar to four and ... 206	5E-52	TZR
contig01611	gi|126632812|emb|CAM56654.1| novel protein (zgc:55818) [*Danio re*... 208	4E-52	O
contig12269	gi|90820032|gb|ABD98773.1| putative GABA-A receptor associated p... 211	3E-52	Z
contig03618	gi|156547544|ref|XP_001607621.1| PREDICTED: similar to ENSANGP00... 209	6E-53	O
contig02452	gi|73962301|ref|XP_537355.2| PREDICTED: similar to Legumain prec... 152	2E-53	O
contig11297	gi|110759191|ref|XP_393334.3| PREDICTED: similar to Myosin heavy... 199	1E-53	Z
contig04274	gi|66541426|ref|XP_624341.1| PREDICTED: similar to Proteasome su... 213	7E-54	O
contig00256	gi|109255261|ref|YP_654538.1| replicase [*Cytoplasmic citrus lepr*... 220	1E-54	WV
contig12280	gi|156367369|ref|XP_001627390.1| predicted protein [*Nematostella*... 216	6E-55	T
contig13090	gi|167683074|gb|ABZ91679.1| actin [*Uloborus diversus*] 218	1E-55	Z
contig01313	gi|91078924|ref|XP_973781.1| PREDICTED: similar to actin [*Tribol*... 218	9E-56	Z
contig12809	gi|149287016|gb|ABR23407.1| putative ubiquitin-conjugating enzym... 219	7E-56	O
contig05900	gi|161788938|dbj|BAF95090.1| 2nd tick legumain [*Haemaphysalis lo*... 199	3E-56	O
contig01948	gi|55140565|gb|AAV41826.1| myosin alkali light chain protein [*Ha*... 223	1E-56	Z
contig01511	gi|114153168|gb|ABI52750.1| signal peptidase complex I [*Argas mo*... 224	4E-57	U
contig06281	gi|91078858|ref|XP_972061.1| PREDICTED: similar to thymus-specif... 224	2E-57	OR
contig07074	gi|193643451|ref|XP_001946772.1| PREDICTED: similar to uridine c... 225	2E-57	TZ
contig04812	gi|110757619|ref|XP_001120141.1| PREDICTED: similar to Arflike a... 225	1E-57	U
contig00721	gi|136050|sp|P15159|TNNC_TACTR Troponin C >gnl|BL_ORD_ID|766147 ... 228	7E-58	T
contig04645	gi|198145382|gb|EAL32343.2| GA13115 [*Drosophila pseudoobscura* ps... 162	7E-58	Z
contig02656	gi|2443346|dbj|BAA22382.1| beta-tubulin [*Halocynthia roretzi*] 226	6E-58	Z
contig03733	gi|148695032|gb|EDL26979.1| proteasome (prosome, macropain) 26S ... 227	3E-58	O
contig09816	gi|110760400|ref|XP_396948.3| PREDICTED: similar to misshapen CG... 228	2E-58	T
contig12602	gi|47213256|emb|CAF92917.1| unnamed protein product [*Tetraodon n*... 232	7E-59	D
contig04217	gi|18031682|gb|AAK31583.1| heat shock protein 70 [*Ambystoma mexi*... 145	7E-59	O
contig03835	gi|148717303|dbj|BAF63637.1| glucose-regulated protein 94 [*Crass*... 229	6E-59	O
contig02455	gi|15428288|gb|AAK97814.1|AF209911_1 glutathione peroxidase [*Ixo*... 229	5E-59	O
contig04556	gi|194385190|dbj|BAG61001.1| unnamed protein product [*Homo sapiens*] 230	3E-59	Z
contig00410	gi|170574536|ref|XP_001892857.1| Oligosaccharyl transferase STT3... 231	1E-59	O
contig00825	gi|156394193|ref|XP_001636711.1| predicted protein [*Nematostella*... 231	1E-59	TR
contig01548	gi|156373877|ref|XP_001629536.1| predicted protein [*Nematostella*... 232	8E-60	O
contig03011	gi|91087761|ref|XP_974991.1| PREDICTED: similar to predicted pro... 236	2E-60	O
contig05653	gi|158299538|ref|XP_319643.4| AGAP008896-PA [*Anopheles gambiae* s... 236	6E-61	UR
contig03208	gi|148717317|dbj|BAF63672.1| protein disulfide isomerase-1 [*Haem*... 241	5E-62	O
contig07241	gi|68161644|emb|CAG44455.1| cAMP-dependent protein kinase cataly... 241	1E-62	T
contig00347	gi|18542438|gb|AAL75583.1|AF467697_1 clathrin-adaptor protein [*D*... 242	1E-62	U
contig04870	gi|183979376|dbj|BAG30740.1| muscle myosin heavy chain [*Papilio* ... 244	7E-63	Z
contig06637	gi|197128441|gb|ACH44939.1| putative proteasome 26S subunit ATPa... 247	2E-64	O

Contig	BLAST result	e value	Class
contig00873	gi|2665388|dbj|BAA23764.1| cyclophilin G [*Tachypleus tridentatus*] 251	5E-65	O
contig00611	gi|195059281|ref|XP_001995602.1| GH17842 [*Drosophila grimshawi*] ... 251	4E-65	O
contig03366	gi|156545571|ref|XP_001607375.1| PREDICTED: similar to proteasom... 252	7E-66	O
contig03909	gi|46370306|gb|AAS89952.1| GroEL [*Bartonella phoceensis*] 185	2E-66	O
contig12625	gi|17647231|ref|NP_523710.1| Calmodulin CG8472-PA, isoform A [*Dr*... 234	1E-66	T
contig12116	gi|182701796|ref|ZP_02955128.1| conserved hypothetical protein [... 182	8E-67	O
contig01081	gi|91089049|ref|XP_969998.1| PREDICTED: similar to AGAP011768-PA... 256	5E-67	T
contig03864	gi|193657249|ref|XP_001950152.1| PREDICTED: similar to rab32 [*Ac*... 259	3E-67	T
contig02379	gi|66513481|ref|XP_394434.2| PREDICTED: similar to Ubiquitin act... 259	1E-67	O
contig00773	gi|189241320|ref|XP_966927.2| PREDICTED: similar to CG5871 CG587... 224	5E-69	O
contig13125	gi|115715524|ref|XP_780580.2| PREDICTED: similar to cathepsin l ... 231	5E-71	O
contig12685	gi|193643435|ref|XP_001945057.1| PREDICTED: similar to transport... 271	3E-71	U
contig01569	gi|91085931|ref|XP_970194.1| PREDICTED: similar to proteasome su... 272	2E-71	O
contig03808	gi|156549531|ref|XP_001602213.1| PREDICTED: similar to ubiquitin... 272	2E-71	O
contig05159	gi|17391080|gb|AAH18459.1| Cct6a protein [*Mus musculus*] 271	1E-71	O
contig03303	gi|112983370|ref|NP_001037628.1| transport protein Sec61 alpha s... 272	5E-72	UO
contig04106	gi|148717305|dbj|BAF63638.1| calnexin [*Crassostrea gigas*] 234	7E-73	O
contig00331	gi|195113909|ref|XP_002001510.1| GI10836 [*Drosophila mojavensis*]... 276	4E-73	U
contig11484	gi|156544337|ref|XP_001607303.1| PREDICTED: similar to CG17927-P... 192	4E-73	Z
contig12438	gi|194757347|ref|XP_001960926.1| GF13608 [*Drosophila ananassae*] ... 244	7E-74	O
contig01172	gi|65332126|gb|AAY42205.1| troponin T [*Haemaphysalis qinghaiensis*] 285	5E-75	Z
contig04987	gi|91090858|ref|XP_967143.1| PREDICTED: similar to annexin B13a ... 286	2E-75	U
contig13301	gi|194354483|gb|ACF54724.1| beta-actin [*Mantichorula semenowi*] 284	1E-75	Z
contig02485	gi|66524917|ref|XP_393112.2| PREDICTED: similar to 26S proteasom... 219	7E-76	O
contig00438	gi|51011504|gb|AAT92161.1| myosin regulatory light chain [Ixodes... 219	1E-76	Z
contig03511	gi|157121273|ref|XP_001659895.1| ran [*Aedes aegypti*] >gnl|BL_ORD... 291	2E-77	U
contig11475	gi|55925548|ref|NP_001007314.1| protein phosphatase 1A, magnesiu... 167	1E-77	T
contig13176	gi|24646906|ref|NP_731941.1| effete CG7425-PA [*Drosophila melano*... 302	2E-80	O
contig13051	gi|57163957|ref|NP_001009202.1| polyubiquitin [*Ovis aries*] >gnl|... 301	2E-80	OR
contig12133	gi|154183745|gb|ABS70713.1| cathepsin L-like cysteine proteinase... 176	1E-80	O
contig02861	gi|66513649|ref|XP_623691.1| PREDICTED: similar to Spectrin alph... 302	7E-81	Z
contig01798	gi|156361252|ref|XP_001625432.1| predicted protein [*Nematostella*... 308	2E-82	O
contig00453	gi|156381354|ref|XP_001632230.1| predicted protein [*Nematostella*... 309	2E-82	TU
contig01747	gi|91085175|ref|XP_971032.1| PREDICTED: similar to ubiquitin con... 313	4E-83	O
contig11327	gi|152205934|dbj|BAF73714.1| valosin containing protein [*Haemaph*... 313	1E-83	O
contig11660	gi|22901939|gb|AAN10129.1| alpha-2-macroglobulin precursor splic... 319	3E-85	O
contig12493	gi|73958240|ref|XP_537920.2| PREDICTED: similar to protein kinas... 323	1E-86	T
contig12243	gi|39725977|gb|AAR29940.1| calreticulin [*Boophilus microplus*] 323	7E-87	O
contig00197	gi|149287010|gb|ABR23404.1| thioredoxin peroxidase [*Ornithodoros*... 326	2E-87	O
contig02000	gi|91087475|ref|XP_967673.1| PREDICTED: similar to 26S protease ... 324	2E-87	O
contig11566	gi|156407426|ref|XP_001641545.1| predicted protein [*Nematostella*... 332	6E-89	U
contig06778	gi|197322816|gb|ACH69159.1| myosin heavy chain [*Bombyx mori*] 339	9E-92	Z
contig00679	gi|189234306|ref|XP_971669.2| PREDICTED: similar to protein disu... 347	1E-93	O
contig11995	gi|13562118|ref|NP_110454.1| low density lipoprotein receptor-re... 188	2E-94	T
contig00399	gi|195972560|emb|CAQ77161.1| histamine release factor [*Dermanyss*... 350	1E-94	DZ
contig02474	gi|67083703|gb|AAY66786.1| mitochondrial truncated thioredoxin-d... 350	9E-95	O
contig00786	gi|109114958|ref|XP_001094505.1| PREDICTED: proteasome 26S non-A... 257	4E-99	O
contig03320	gi|94448674|emb|CAI91575.1| cathepsin L2 [*Lubomirskia baicalensis*] 369	1E-100	O
contig00670	gi|72008176|ref|XP_780713.1| PREDICTED: similar to cathepsin L [... 377	1E-102	O
contig04310	gi|158287073|ref|XP_309120.3| AGAP000935-PA [*Anopheles gambiae* s... 305	1E-104	O
contig11625	gi|170030742|ref|XP_001843247.1| proteasome subunit alpha type 6... 384	1E-105	O

Contig	BLAST result	e value	Class
contig13343	gi|156553657|ref|XP_001600604.1| PREDICTED: similar to ENSANGP00... 386	1E-105	O
contig02395	gi|195390229|ref|XP_002053771.1| GJ24070 [*Drosophila virilis*] >g... 387	1E-106	O
contig00995	gi|48097086|ref|XP_391841.1| PREDICTED: similar to 14-3-3-like p... 254	1E-108	O
contig00320	gi|83308265|emb|CAJ44440.1| tropomyosin [*Dermanyssus gallinae*] 410	1E-112	Z
contig11792	gi|146217394|gb|ABQ10739.1| cathepsin L [*Penaeus monodon*] 413	1E-113	O
contig12527	gi|157131971|ref|XP_001662386.1| prohibitin [*Aedes aegypti*] >gnl... 411	1E-113	O
contig13094	gi|28932702|gb|AAO60045.1| midgut cysteine proteinase 2 [*Rhipice*... 416	1E-118	O
contig01604	gi|110734697|gb|ABG88953.1| elongation factor-1 gamma [*Diguetia* ... 434	1E-119	O
contig12925	gi|50539738|ref|NP_001002335.1| karyopherin alpha 2 (RAG cohort ... 439	1E-121	U
contig00293	gi|83319201|dbj|BAE53722.1| aspartic protease [*Haemaphysalis lon*... 451	1E-125	O
contig03441	gi|18542438|gb|AAL75583.1|AF467697_1 clathrin-adaptor protein [*D*... 450	1E-125	U
contig02172	gi|170015974|ref|NP_001116163.1| thioredoxin reductase 2 [*Gallus*... 330	1E-129	O
contig11932	gi|118102310|ref|XP_419249.2| PREDICTED: hypothetical protein [*G*... 286	1E-130	Z
contig03245	gi|74211237|dbj|BAE37687.1| unnamed protein product [*Mus musculus*] 431	1E-132	T
contig02198	gi|195128571|ref|XP_002008736.1| GI11652 [*Drosophila mojavensis*]... 353	1E-147	O
contig00018	gi|149287130|gb|ABR23464.1| G protein beta subunit-like protein ... 525	1E-147	T
contig11091	gi|111380719|gb|ABH09735.1| heat shock cognate 70 protein [*Trich*... 358	1E-149	O
contig12961	gi|60302774|ref|NP_001012581.1| chaperonin containing TCP1, subu... 308	1E-152	O
contig00224	gi|39725965|gb|AAR29934.1| calreticulin [*Amblyomma cooperi*] 546	1E-153	O
contig11293	gi|148717315|dbj|BAF63671.1| protein disulfide isomerase-2 [*Haem*... 385	1E-160	O
contig01323	gi|87042250|gb|ABD16189.1| protein disulfide isomerase [*Amblyomm*... 333	1E-173	O
contig01178	gi|115748959|ref|XP_784913.2| PREDICTED: similar to Chaperonin c... 617	1E-175	O
contig00147	gi|150034859|gb|ABR66910.1| heat shock protein 90 [*Metapenaeus e*... 1038	0E+00	O
contig00200	gi|112982828|ref|NP_001036892.1| heat shock cognate protein [*Bom*... 969	0	O
contig00472	gi|66547450|ref|XP_392899.2| PREDICTED: similar to 60 kDa heat s... 819	0	O
contig00639	gi|156540734|ref|XP_001599525.1| PREDICTED: similar to CG8542-PA... 883	0	O
contig02359	gi|91086235|ref|XP_966692.1| PREDICTED: similar to transitional ... 818	0	O
contig02363	gi|29825445|gb|AAO92283.1| 26S proteasome regulatory subunit [*De*... 484	0	O
contig12143	gi|158147455|emb|CAL68992.1| heat shock protein 70 kDa [*Xantho i*... 664	0	O
contig00452	gi|170034793|ref|XP_001845257.1| conserved hypothetical protein ... 1177	0	OR
contig12705	gi|189208430|ref|XP_001940548.1| laccase-1 precursor [*Pyrenophor*... 26	0	T
contig11712	gi|183987114|gb|ACC65584.1| paramyosin [*Sarcoptes scabiei*] 634	0	Z
contig13015	gi|10242160|gb|AAG15315.1|AF255553_1 beta tubulin [*Notothenia co*... 680	0	Z
contig13080	gi|45269081|gb|AAS55945.1| actin [*Ornithodoros moubata*] 742	0	Z
contig13115	gi|83318905|emb|CAJ38791.1| alpha tubulin protein [*Platynereis d*... 848	0	Z
contig02456	No hits found		D
contig09106	No hits found		D
contig00621	No hits found		M
contig02188	No hits found		M
contig11756	No hits found		M
contig09992	No hits found		N
contig01187	No hits found		O
contig03279	No hits found		O
contig04579	No hits found		O
contig07346	No hits found		O
contig07744	No hits found		O
contig08752	No hits found		O
contig09130	No hits found		O
contig09290	No hits found		O
contig09581	No hits found		O
contig10748	No hits found		O

Contig	BLAST result	e value	Class
contig10860	No hits found		O
contig11543	No hits found		O
contig00648	No hits found		OT
contig09960	No hits found		OW
contig01040	No hits found		T
contig01330	No hits found		T
contig01335	No hits found		T
contig01594	No hits found		T
contig01671	No hits found		T
contig01899	No hits found		T
contig02561	No hits found		T
contig03545	No hits found		T
contig03694	No hits found		T
contig03866	No hits found		T
contig04023	No hits found		T
contig04592	No hits found		T
contig04616	No hits found		T
contig05428	No hits found		T
contig05871	No hits found		T
contig05979	No hits found		T
contig06386	No hits found		T
contig06400	No hits found		T
contig06413	No hits found		T
contig06993	No hits found		T
contig07114	No hits found		T
contig07770	No hits found		T
contig08038	No hits found		T
contig08166	No hits found		T
contig08452	No hits found		T
contig08603	No hits found		T
contig09120	No hits found		T
contig09320	No hits found		T
contig09632	No hits found		T
contig10700	No hits found		T
contig10772	No hits found		T
contig10852	No hits found		T
contig11080	No hits found		T
contig11632	No hits found		T
contig11825	No hits found		T
contig12174	No hits found		T
contig12211	No hits found		T
contig12901	No hits found		T
contig04167	No hits found		TR
contig01654	No hits found		TU
contig06476	No hits found		TZ
contig07019	No hits found		TZ
contig01570	No hits found		U
contig05806	No hits found		U
contig05835	No hits found		U
contig10411	No hits found		U
contig11174	No hits found		U

Contig	BLAST result	e value	Class
contig12431	No hits found		U
contig12636	No hits found		U
contig02072	No hits found		W
contig03523	No hits found		W
contig04608	No hits found		W
contig06158	No hits found		W
contig00979	No hits found		Z
contig02190	No hits found		Z
contig02636	No hits found		Z
contig03242	No hits found		Z
contig05659	No hits found		ZU

Cellular processes and signaling and Metabolism

Contig	BLAST result	e value	Class
contig01361	gi|19172975|ref|NP_597526.1| GOLGI MEMBRANE PROTEIN [*Encephalito*... 34	8.10	GOU
contig04295	gi|183985269|ref|YP_001853560.1| osmoprotectant transport ATP-bi... 33	7.40	TG
contig12716	gi|167526593|ref|XP_001747630.1| predicted protein [*Monosiga bre*... 33	7.10	PT
contig09594	gi|170065803|ref|XP_001868034.1| activin receptor type II [*Culex*... 34	4.30	PT
contig00895	gi|195341800|ref|XP_002037493.1| GM18293 [*Drosophila sechellia*] ... 34	4.20	OC
contig00657	gi|94264824|ref|ZP_01288600.1| Oxidoreductase FAD/NAD(P)-binding... 34	3.20	PT
contig03144	gi|148557003|ref|YP_001264585.1| hypothetical protein Swit_4104 ... 34	3.20	IT
contig06119	gi|116056702|emb|CAL52991.1| Zn-binding protein Push (ISS) [*Ostr*... 34	3.20	GOU
contig01658	gi|55962840|emb|CAI17143.1| calcium channel, voltage-dependent, ... 35	2.50	PT
contig01334	gi|168044085|ref|XP_001774513.1| predicted protein [*Physcomitrel*... 35	1.50	GOT
contig00243	gi|187478146|ref|YP_786170.1| LysR-family transcriptional regula... 36	1.10	PT
contig05456	gi|15341234|dbj|BAB63947.1| Ser/Thr protein phosphatase [*Caenorh*... 37	0.80	GT
contig02753	gi|196014576|ref|XP_002117147.1| hypothetical protein TRIADDRAFT... 37	0.66	NI
contig13213	gi|195112951|ref|XP_002001035.1| GI22201 [*Drosophila mojavensis*]... 37	0.65	GO
contig06251	gi|195171619|ref|XP_002026601.1| GL11802 [*Drosophila persimilis*]... 38	0.63	PT
contig07412	gi|170061586|ref|XP_001866297.1| endophilin a [*Culex quinquefasc*... 38	0.29	IT
contig12340	gi|171685035|ref|XP_001907459.1| unnamed protein product [*Podosp*... 38	0.29	OE
contig05729	gi|66826615|ref|XP_646662.1| hypothetical protein DDBDRAFT_01909... 38	0.23	PT
contig10284	gi|157106778|ref|XP_001649477.1| o-linked n-acetylglucosamine tr... 38	0.22	GOT
contig13200	gi|111220888|ref|YP_711682.1| 1-hydroxy-2-methyl-2-(E)-butenyl 4... 43	0.05	EO
contig13357	gi|162149485|ref|YP_001603946.1| HlyD family secretion protein [... 43	0.04	PT
contig01068	gi|156545822|ref|XP_001606135.1| PREDICTED: similar to LD41494p ... 50	4E-05	OC
contig05888	gi|160333701|ref|NP_001103872.1| UDP-glucose dehydrogenase [*Dani*... 54	4E-06	GT
contig00433	gi|91090053|ref|XP_968775.1| PREDICTED: similar to neural stem c... 54	3E-06	IOT
contig05365	gi|73951761|ref|XP_536218.2| PREDICTED: similar to Alpha-2-macro... 57	9E-07	OUTIV
contig10993	gi|118792081|ref|XP_001238258.1| AGAP012412-PA [Anopheles gambia... 57	6E-07	IM
contig11601	gi|193596507|ref|XP_001944612.1| PREDICTED: similar to AGAP00572... 59	2E-07	EO
contig06102	gi|147906564|ref|NP_001086043.1| MGC81801 protein [*Xenopus laevi*... 60	7E-08	GMO
contig01197	gi|158294663|ref|XP_556379.3| AGAP005728-PA [*Anopheles gambiae* s... 63	7E-09	EO
contig10084	gi|156552278|ref|XP_001600652.1| PREDICTED: similar to Na/Ca exc... 64	4E-09	PT
contig08219	gi|195120496|ref|XP_002004760.1| GI20092 [*Drosophila mojavensis*]... 64	3E-09	OE
contig03559	gi|156404336|ref|XP_001640363.1| predicted protein [*Nematostella*... 53	2E-10	PT
contig08759	gi|126352610|ref|NP_001075382.1| transferrin receptor [*Equus cab*... 72	1E-11	OPR
contig04697	gi|190339135|gb|AAI63301.1| Unknown (protein for MGC:194608) [Da... 74	5E-12	GMO
contig05197	gi|47215938|emb|CAF96340.1| unnamed protein product [*Tetraodon n*... 71	5E-14	GOU
contig05762	gi|193674143|ref|XP_001949237.1| PREDICTED: similar to CG9342 CG... 81	3E-14	IU
contig03934	gi|170573720|ref|XP_001892576.1| Voltage-gated calcium channel, ... 88	2E-16	PT
contig10389	gi|156546630|ref|XP_001602950.1| PREDICTED: similar to retinoid-... 89	9E-17	OE
contig04969	gi|156544540|ref|XP_001607806.1| PREDICTED: similar to CG5269-PA... 90	5E-17	IT
contig11936	gi|158294663|ref|XP_556379.3| AGAP005728-PA [*Anopheles gambiae* s... 80	6E-20	EO
contig10249	gi|167525465|ref|XP_001747067.1| predicted protein [*Monosiga bre*... 101	2E-20	IOVE
contig05012	gi|118092771|ref|XP_426509.2| PREDICTED: similar to polycystin-L... 102	1E-20	PT
contig01270	gi|189240987|ref|XP_001809298.1| PREDICTED: similar to potassium... 104	3E-21	PT
contig01418	gi|156550327|ref|XP_001600741.1| PREDICTED: similar to pra1 prot... 105	1E-21	ET
contig05889	gi|193627424|ref|XP_001947931.1| PREDICTED: similar to endophili... 109	6E-23	IT
contig12172	gi|154500170|ref|ZP_02038208.1| hypothetical protein BACCAP_0383... 116	5E-25	PT
contig00919	gi|114051728|ref|NP_001040176.1| NADH dehydrogenase (ubiquinone)... 120	2E-25	CD
contig03190	gi|67083971|gb|AAY66920.1| possible succinate-ubiquinone reducta... 128	4E-28	CU
contig06630	gi|170573720|ref|XP_001892576.1| Voltage-gated calcium channel, ... 132	9E-30	PT

Contig	BLAST result	e value	Class
contig03031	gi|149634049|ref|XP_001511641.1| PREDICTED: similar to putative ... 133	4E-30	OE
contig04546	gi|110765015|ref|XP_624669.2| PREDICTED: similar to Serine/threo... 140	3E-32	GT
contig02211	gi|158290203|ref|XP_311781.3| AGAP001058-PA [*Anopheles gambiae* s... 147	3E-34	IO
contig04478	gi|17559180|ref|NP_504655.1| F46E10.9 [*Caenorhabditis elegans*] >... 204	1E-50	OC
contig01195	gi|198149515|gb|EAL31307.2| GA10064 [*Drosophila pseudoobscura* ps... 262	2E-68	EO
contig11372	gi|193631919|ref|XP_001951188.1| PREDICTED: similar to Myotubula... 286	8E-76	IU
contig12048	gi|195490422|ref|XP_002093133.1| GE21157 [*Drosophila yakuba*] >gn... 106	4E-96	EO
contig11836	gi|40548304|ref|NP_954972.1| carboxypeptidase, vitellogenic-like... 303	1E-122	OE
contig00933	No hits found		GM
contig04448	No hits found		PT
contig07497	No hits found		GM
contig09113	No hits found		PET
contig09561	No hits found		IT

Information storage and processing

Contig	BLAST result	e value	Class
contig05124	gi|194374455|dbj|BAG57123.1| unnamed protein product [*Homo sapiens*] 33	9.600	B
contig02671	gi|121603509|ref|YP_980838.1| FAD linked oxidase domain-containi... 33	9.500	L
contig05610	gi|91777106|ref|YP_546862.1| protein translocase subunit yidC [M... 33	9.500	K
contig08230	gi|189518188|ref|XP_683042.3| PREDICTED: Scm-like with four mbt ... 33	9.500	K
contig09627	gi|89071147|ref|ZP_01158342.1| Siderophore-interacting protein [... 33	9.500	J
contig10633	gi|149923996|ref|ZP_01912380.1| hypothetical protein PPSIR1_0653... 33	9.500	J
contig01810	gi|152978930|ref|YP_001344559.1| formate dehydrogenase, alpha su... 33	9.400	J
contig04050	gi|160013866|sp|Q5AB15.2|IPI3_CANAL Pre-rRNA-processing protein ... 33	9.400	K
contig04103	gi|157104671|ref|XP_001648516.1| mediator complex, subunit, puta... 33	9.400	KL
contig04545	gi|195478403|ref|XP_002100505.1| GE17103 [*Drosophila yakuba*] >gn... 33	9.400	L
contig13113	gi|47458915|ref|YP_015777.1| putative rRNA methylase [*Mycoplasma*... 33	9.400	K
contig01011	gi|20072170|gb|AAH26177.1| MGC27348 protein [*Homo sapiens*] 33	9.300	J
contig13154	gi|42559896|sp|Q9U5M4|TPM2_PODCA Tropomyosin-2 >gnl|BL_ORD_ID|85... 33	8.200	K
contig05004	gi|195035285|ref|XP_001989108.1| GH10228 [*Drosophila grimshawi*] ... 33	7.300	K
contig06330	gi|84500302|ref|ZP_00998568.1| hypothetical protein OB2597_10179... 33	7.300	K
contig06343	gi|67521968|ref|XP_659045.1| hypothetical protein AN1441.2 [*Aspe*... 33	7.300	A
contig09697	gi|192759134|gb|EDV73629.1| lava lamp protein [*Culex quinquefasc*... 33	7.300	A
contig00038	gi|194225212|ref|XP_001490986.2| PREDICTED: similar to Jun dimer... 33	7.200	K
contig02382	gi|119899016|ref|YP_934229.1| flagellar biosynthesis protein Fli... 33	7.200	K
contig03226	gi|32398950|emb|CAD98415.1| PAN domain protein [*Cryptosporidium* ... 33	7.200	A
contig03976	gi|123478136|ref|XP_001322232.1| hypothetical protein TVAG_12818... 33	7.200	J
contig05651	gi|17536939|ref|NP_496560.1| Y17G7B.12 [*Caenorhabditis elegans*] ... 33	7.200	L
contig06092	gi|126342708|ref|XP_001365584.1| PREDICTED: similar to histidine... 33	7.200	K
contig06727	gi|125538332|gb|EAY84727.1| hypothetical protein OsI_005960 [Ory... 33	7.200	K
contig07061	gi|19547871|gb|AAL87457.1| serine/threonine protein kinase pk23 ... 33	7.200	J
contig10055	gi|120602907|ref|YP_967307.1| DNA polymerase III, delta [Desulfo... 33	7.200	A
contig03002	gi|62859687|ref|NP_001016716.1| hypothetical protein LOC549470 [... 33	7.100	B
contig12996	gi|195436782|ref|XP_002066334.1| GK18240 [*Drosophila willistoni*]... 33	7.100	J
contig00056	gi|194748797|ref|XP_001956829.1| GF10124 [*Drosophila ananassae*] ... 33	5.600	L
contig02842	gi|170043937|ref|XP_001849622.1| conserved hypothetical protein ... 33	5.600	B
contig09941	gi|164660100|ref|XP_001731173.1| hypothetical protein MGL_1356 [... 33	5.600	L
contig10035	gi|74003268|ref|XP_857479.1| PREDICTED: similar to Translation i... 33	5.600	J
contig11353	gi|149190975|ref|ZP_01869237.1| hypothetical protein VSAK1_26575... 33	5.600	K
contig12382	gi|195043683|ref|XP_001991668.1| GH11938 [*Drosophila grimshawi*] ... 33	5.600	K
contig02300	gi|121710258|ref|XP_001272745.1| HECT domain protein [*Aspergillu*... 33	5.500	J
contig05646	gi|194227065|ref|XP_001915913.1| PREDICTED: similar to Zinc fing... 33	5.500	K
contig07387	gi|161761242|pdb|3BGV|A Chain A, The Crystals Structure Of Mrna ... 33	5.500	A
contig08134	gi|45359004|ref|NP_988561.1| molybdopterin-guanine dinucleotide ... 33	5.500	J
contig08734	gi|147835330|emb|CAN67672.1| hypothetical protein [*Vitis vinifera*] 33	5.500	J
contig12081	gi|163700748|ref|ZP_02119601.1| glycosyl transferase, group 1 [*M*... 33	5.500	K
contig12141	gi|167034074|ref|YP_001669305.1| ArsR family transcriptional reg... 33	5.500	K
contig10948	gi|149632421|ref|XP_001510539.1| PREDICTED: hypothetical protein... 33	5.400	L
contig12181	gi|195567395|ref|XP_002107246.1| GD17354 [*Drosophila simulans*] >... 32	5.400	K
contig12690	gi|67772310|gb|AAY79377.1| hch [*Fusarium proliferatum*] 34	4.800	B
contig00196	gi|66816547|ref|XP_642283.1| putative extracellular matrix prote... 34	4.300	K
contig00987	gi|161870105|ref|YP_001599275.1| hypothetical protein NMCC_1144 ... 34	4.300	K
contig01117	gi|149586739|ref|XP_001516059.1| PREDICTED: similar to p28 ING5 ... 34	4.300	B
contig01339	gi|198151499|gb|EAL30533.2| GA16795 [*Drosophila pseudoobscura* ps... 34	4.300	J
contig03724	gi|71409575|ref|XP_807125.1| mucin-associated surface protein (M... 34	4.300	K

Contig	BLAST result	e value	Class
contig05350	gi|126541150|emb|CAM45911.1| novel protein [*Mus musculus*] 34	4.300	B
contig08499	gi|170096352|ref|XP_001879396.1| predicted protein [*Laccaria bic*... 34	4.300	L
contig09937	gi|19112932|ref|NP_596140.1| 40S ribosomal protein S0A (p40) [*Sc*... 34	4.300	J
contig12545	gi|116003975|ref|NP_001070347.1| smoothelin [*Bos taurus*] >gnl|BL... 34	4.300	K
contig00511	gi|170114445|ref|XP_001888419.1| predicted protein [*Laccaria bic*... 34	4.200	AR
contig00829	gi|148234927|ref|NP_001083333.1| hypothetical protein LOC398871 ... 34	4.200	A
contig01345	gi|188590827|ref|YP_001795427.1| putative HIPPURATE HYDROLASE [*C*... 34	4.200	J
contig01408	gi|126731378|ref|ZP_01747185.1| glucose-6-phosphate isomerase [S... 34	4.200	B
contig01691	gi|45387885|ref|NP_991303.1| BarH-like 2 [*Danio rerio*] >gnl|BL_O... 34	4.200	K
contig02783	gi|195487951|ref|XP_002092108.1| GE11845 [*Drosophila yakuba*] >gn... 34	4.200	K
contig02868	gi|194665922|ref|XP_875648.3| PREDICTED: similar to Splicing fac... 34	4.200	A
contig11473	gi|167524793|ref|XP_001746732.1| predicted protein [*Monosiga bre*... 34	4.200	K
contig12764	gi|118088246|ref|XP_419583.2| PREDICTED: similar to Cog2 protein... 34	4.200	K
contig05851	gi|115379616|ref|ZP_01466701.1| hypothetical protein STIAU_8201 ... 34	4.100	K
contig02542	gi|58583085|ref|YP_202101.1| abortive infection bacteriophage re... 35	3.500	K
contig02555	gi|149634900|ref|XP_001509468.1| PREDICTED: similar to serine/ar... 34	3.300	AR
contig04247	gi|195391078|ref|XP_002054190.1| GJ24302 [*Drosophila virilis*] >g... 34	3.300	BK
contig11452	gi|73959522|ref|XP_853674.1| PREDICTED: similar to Endonuclease ... 34	3.300	L
contig12355	gi|68086434|gb|AAH93108.2| Programmed cell death 7 [*Danio rerio*] 34	3.300	BK
contig02516	gi|190894294|ref|YP_001984588.1| oxygen-independent coprophorphy... 34	3.200	A
contig03691	gi|157767564|ref|XP_001667262.1| Hypothetical protein CBG16055 [... 34	3.200	A
contig04508	gi|85076351|ref|XP_955914.1| hypothetical protein NCU04456 [*Neur*... 34	3.200	A
contig05279	gi|104782107|ref|YP_608605.1| non-ribosomal peptide synthetase, ... 34	3.200	K
contig05747	gi|60117201|ref|YP_209535.1| hypothetical chloroplast RF2 [*Huper*... 34	3.200	K
contig06762	gi|18411789|ref|NP_567106.1| C2 domain-containing protein [*Arabi*... 34	3.200	L
contig08846	gi|17557646|ref|NP_504838.1| C05C8.5 [*Caenorhabditis elegans*] >g... 34	3.200	L
contig00265	gi|195151592|ref|XP_002016723.1| GL10349 [*Drosophila persimilis*]... 35	2.500	AR
contig01677	gi|195996431|ref|XP_002108084.1| hypothetical protein TRIADDRAFT... 35	2.500	L
contig01824	gi|110777050|ref|XP_001123116.1| PREDICTED: similar to Tetratric... 35	2.500	K
contig03898	gi|195116507|ref|XP_002002796.1| GI11029 [*Drosophila mojavensis*]... 35	2.500	A
contig05998	gi|198251761|gb|EDY76076.1| hypothetical protein OA307_1098 [Oct... 35	2.500	L
contig06606	gi|159486775|ref|XP_001701413.1| phosphofructokinase [*Chlamydomo*... 35	2.500	B
contig07381	gi|114550513|ref|XP_001143253.1| PREDICTED: similar to bHLH fact... 35	2.500	K
contig07473	gi|195059412|ref|XP_001995632.1| GH17861 [*Drosophila grimshawi*] ... 35	2.500	B
contig08434	gi|63054602|ref|NP_594222.2| mitochondrial ribosomal protein sub... 35	2.500	J
contig08454	gi|66805733|ref|XP_636588.1| hypothetical protein DDBDRAFT_01880... 35	2.500	K
contig09018	gi|148239740|ref|YP_001225127.1| hypothetical protein SynWH7803_... 35	2.500	A
contig09254	gi|170036541|ref|XP_001846122.1| conserved hypothetical protein ... 35	2.500	BK
contig12748	gi|114675075|ref|XP_001146978.1| PREDICTED: similar to transform... 35	2.500	K
contig07765	gi|115631503|ref|XP_793321.2| PREDICTED: similar to Nuclear rece... 35	2.400	K
contig10530	gi|146310031|ref|YP_001175105.1| methyl-accepting chemotaxis sen... 35	2.400	K
contig03207	gi|118360979|ref|XP_001013720.1| hypothetical protein TTHERM_004... 35	2.300	A
contig13260	gi|6012073|gb|AAB82017.2| microfilarial sheath protein SHP3 [Lit... 38	2.100	L
contig02454	gi|86156144|gb|ABC86790.1| ribonucleotide reductase alpha subuni... 35	1.900	K
contig06574	gi|157770618|ref|XP_001677129.1| Hypothetical protein CBG16774 [... 35	1.900	K
contig08409	gi|76154922|gb|AAX26319.2| SJCHGC08564 protein [*Schistosoma japo*... 35	1.900	B
contig08652	gi|37525042|ref|NP_928386.1| hypothetical protein plu1057 [Photo... 35	1.900	A
contig09477	gi|34597230|gb|AAQ77190.1| elongation factor 2 [*Sphaerotherium p*... 35	1.900	J
contig10602	gi|6015452|dbj|BAA85169.1| dopamine receptor D4 [*Hylobates agilis*] 35	1.900	BK
contig12695	gi|149409316|ref|XP_001509568.1| PREDICTED: similar to hCG199415... 35	1.900	L
contig13316	gi|170089261|ref|XP_001875853.1| predicted protein [*Laccaria bic*... 35	1.900	J

Contig	BLAST result	e value	Class
contig10854	gi|66519677|ref|XP_393827.2| PREDICTED: similar to Big brother C... 29	1.700	K
contig03917	gi|47204164|emb|CAG13473.1| unnamed protein product [*Tetraodon n*... 35	1.500	K
contig05206	gi|72162076|ref|YP_289733.1| hypothetical protein Tfu_1675 [*Ther*... 35	1.500	K
contig11921	gi|153816219|ref|ZP_01968887.1| hypothetical protein RUMTOR_0246... 35	1.500	K
contig12274	gi|21357063|ref|NP_649548.1| Snm1 CG10018-PA [*Drosophila melanog*... 35	1.500	L
contig12946	gi|42628974|ref|ZP_00154525.1| COG1629: Outer membrane receptor ... 37	1.500	L
contig13064	gi|94496764|ref|ZP_01303339.1| type III secretion system ATPase ... 35	1.500	K
contig13119	gi|116059360|emb|CAL55067.1| unnamed protein product [*Ostreococc*... 35	1.500	A
contig02185	gi|156397119|ref|XP_001637739.1| predicted protein [*Nematostella*... 35	1.400	K
contig04417	gi|169211998|ref|XP_001719409.1| PREDICTED: hypothetical protein... 35	1.400	L
contig05176	gi|49475135|ref|YP_033176.1| Phage protein gp17 [*Bartonella hens*... 35	1.400	BK
contig02250	gi|72388412|ref|XP_844630.1| hypothetical protein, conserved [*Tr*... 38	1.300	J
contig13234	gi|159038238|ref|YP_001537491.1| cytochrome P450 [*Salinispora ar*... 37	1.300	J
contig01829	gi|115487600|ref|NP_001066287.1| Os12g0174300 [*Oryza sativa* (*jap*... 34	1.200	K
contig00372	gi|148677083|gb|EDL09030.1| microtubule-associated protein 4, is... 39	1.100	BK
contig05128	gi|149737471|ref|XP_001498017.1| PREDICTED: similar to ATP-depen... 36	1.100	A
contig06548	gi|116630025|ref|YP_815197.1| Signal transduction histidine kina... 36	1.100	K
contig06783	gi|154275406|ref|XP_001538554.1| E3 ubiquitin--protein ligase pu... 36	1.100	K
contig08272	gi|194903420|ref|XP_001980865.1| GG17394 [*Drosophila erecta*] >gn... 36	1.100	J
contig09546	gi|194374955|dbj|BAG62592.1| unnamed protein product [*Homo sapiens*] 36	1.100	A
contig11026	gi|149052886|gb|EDM04703.1| nuclear receptor co-repressor 1, iso... 36	1.100	K
contig12267	gi|194223682|ref|XP_001914731.1| PREDICTED: bromodomain containi... 36	1.100	K
contig12437	gi|47213666|emb|CAF95619.1| unnamed protein product [*Tetraodon n*... 36	1.100	K
contig03754	gi|125600956|gb|EAZ40532.1| hypothetical protein OsJ_024015 [*Ory*... 36	0.860	A
contig06389	gi|116668953|ref|YP_829886.1| hypothetical protein Arth_0385 [*Ar*... 36	0.860	J
contig06715	gi|120603669|ref|YP_968069.1| hypothetical protein Dvul_2630 [*De*... 36	0.860	K
contig04224	gi|71004404|ref|XP_756868.1| hypothetical protein UM00721.1 [*Ust*... 36	0.850	L
contig11331	gi|110289435|gb|AAP54676.2| methyltransferase family protein, ex... 36	0.850	A
contig12149	gi|168227042|ref|ZP_02652273.1| hypothetical protein Sententeric... 36	0.850	J
contig12247	gi|154332073|ref|XP_001561853.1| microtubule-associated protein,... 36	0.850	A
contig12401	gi|66824905|ref|XP_645807.1| hypothetical protein DDBDRAFT_02029... 36	0.850	L
contig05798	gi|197246418|gb|AAI68833.1| Unknown (protein for IMAGE:8859384) ... 36	0.840	J
contig11944	gi|91084937|ref|XP_971098.1| PREDICTED: similar to Spt5 CG7626-P... 36	0.840	K
contig02769	gi|77359943|ref|YP_339518.1| translation initiation factor IF-2 ... 39	0.680	A
contig06082	gi|126335335|ref|XP_001371550.1| PREDICTED: similar to splicing ... 37	0.660	A
contig10584	gi|39975039|ref|XP_368910.1| predicted protein [*Magnaporthe gris*... 37	0.660	K
contig05949	gi|115707349|ref|XP_001199817.1| PREDICTED: similar to retinitis... 37	0.650	K
contig12157	gi|159466738|ref|XP_001691555.1| predicted protein [*Chlamydomona*... 37	0.650	A
contig01071	gi|134280916|ref|ZP_01767626.1| hypothetical protein BURPS305_37... 39	0.540	A
contig06703	gi|152983013|ref|YP_001355143.1| Tn7-like transposition protein ... 37	0.510	J
contig11652	gi|77553198|gb|ABA95994.1| hypothetical protein LOC_Os12g08490 [... 37	0.510	A
contig02192	gi|91080713|ref|XP_975329.1| PREDICTED: similar to bicoid stabil... 37	0.500	A
contig03547	gi|164427354|ref|XP_956464.2| hypothetical protein NCU03355 [*Neu*... 37	0.500	K
contig04856	gi|194671056|ref|XP_001788407.1| PREDICTED: similar to YLP motif... 37	0.500	BK
contig08106	gi|6225541|sp|O77210.1|IF4E_APLCA Eukaryotic translation initiat... 37	0.500	J
contig00607	gi|193652714|ref|XP_001950832.1| PREDICTED: similar to predicted... 38	0.490	A
contig07088	gi|195043294|ref|XP_001991590.1| GH12742 [*Drosophila grimshawi*] ... 35	0.440	J
contig01333	gi|115471039|ref|NP_001059118.1| Os07g0195900 [*Oryza sativa* (*jap*... 39	0.420	B
contig04598	gi|193643332|ref|XP_001942760.1| PREDICTED: similar to AGAP00225... 37	0.390	L
contig11064	gi|153815791|ref|ZP_01968459.1| hypothetical protein RUMTOR_0203... 37	0.390	K
contig04693	gi|159478833|ref|XP_001697505.1| ubiquitin-protein ligase [*Chlam*... 37	0.380	K

Contig	BLAST result	e value	Class
contig05853	gi|56118378|ref|NP_001008144.1| La ribonucleoprotein domain fami... 37	0.380	L
contig13358	gi|195402857|ref|XP_002060016.1| GJ15500 [*Drosophila virilis*] >g... 37	0.380	K
contig06914	gi|195115399|ref|XP_002002244.1| GI17279 [*Drosophila mojavensis*]... 38	0.340	K
contig07075	gi|147679178|ref|YP_001213393.1| CTP synthetase [*Pelotomaculum t*... 38	0.300	K
contig02638	gi|47217572|emb|CAG02499.1| unnamed protein product [*Tetraodon n*... 38	0.290	J
contig06142	gi|77548411|gb|ABA91208.1| expressed protein [*Oryza sativa* (*japo*... 38	0.290	A
contig08713	gi|124360603|gb|ABN08602.1| Phosphoinositide-binding clathrin ad... 38	0.290	K
contig06044	gi|148690326|gb|EDL22273.1| serine/arginine repetitive matrix 2 ... 39	0.230	A
contig00560	gi|193582536|ref|XP_001946023.1| PREDICTED: similar to CG11486 C... 38	0.220	A
contig05453	gi|156555067|ref|XP_001604099.1| PREDICTED: similar to smooth [N... 38	0.220	A
contig11725	gi|114053047|ref|NP_001040027.1| glutamyl-tRNA(Gln) amidotransfe... 38	0.220	L
contig08306	gi|145252814|ref|XP_001397920.1| hypothetical protein An16g06160... 29	0.210	K
contig11553	gi|194207332|ref|XP_001915885.1| PREDICTED: Fanconi anemia, comp... 40	0.160	L
contig12900	gi|158337389|ref|YP_001518564.1| type I secretion target repeat-... 41	0.150	B
contig06496	gi|189491618|ref|NP_001121625.1| timeless [*Ovis aries*] >gnl|BL_O... 39	0.130	L
contig08268	gi|37220738|gb|AAQ89710.1| rhoptry associated membrane antigen [... 39	0.130	J
contig03293	gi|66806449|ref|XP_636947.1| leucine-rich repeat-containing prot... 39	0.100	K
contig08061	gi|169616167|ref|XP_001801499.1| hypothetical protein SNOG_11257... 39	0.100	B
contig12391	gi|37521692|ref|NP_925069.1| hypothetical protein gll2123 [*Gloeo*... 39	0.100	K
contig04052	gi|195380001|ref|XP_002048759.1| GJ21226 [*Drosophila virilis*] >g... 39	0.099	J
contig08298	gi|49256131|gb|AAH73031.1| Unknown (protein for MGC:82639) [*Xeno*... 40	0.078	A
contig09224	gi|157953923|ref|YP_001498814.1| hypothetical protein AR158_C733... 40	0.078	A
contig03246	gi|156376942|ref|XP_001630617.1| predicted protein [*Nematostella*... 40	0.077	J
contig04572	gi|157116290|ref|XP_001652808.1| hypothetical protein AaeL_AAEL0... 40	0.077	K
contig03717	gi|76156698|gb|AAX27852.2| SJCHGC03298 protein [*Schistosoma japo*... 40	0.063	J
contig06684	gi|141795258|gb|AAI39520.1| Im:7151572 protein [*Danio rerio*] 40	0.059	A
contig01075	gi|149028968|gb|EDL84287.1| rCG63087 [*Rattus norvegicus*] 40	0.058	A
contig07909	gi|54038332|gb|AAH84673.1| LOC495249 protein [*Xenopus laevis*] 40	0.058	AR
contig03179	gi|1237202|emb|CAA65612.1| histidine kinase [*Dictyostelium disco*... 39	0.055	L
contig02570	gi|158315983|ref|YP_001508491.1| alpha/beta hydrolase domain-con... 40	0.045	J
contig10798	gi|115699072|ref|XP_781418.2| PREDICTED: similar to DNA polymera... 40	0.045	L
contig09757	gi|156552292|ref|XP_001600496.1| PREDICTED: similar to ENSANGP00... 40	0.044	J
contig11253	gi|193216773|ref|YP_002000015.1| massive surface protein MspC [*M*... 44	0.037	L
contig12095	gi|170040091|ref|XP_001847845.1| maternal tudor protein [*Culex q*... 44	0.035	K
contig03099	gi|195452550|ref|XP_002073403.1| GK14107 [*Drosophila willistoni*]... 41	0.034	K
contig03382	gi|148702332|gb|EDL34279.1| DEAD (Asp-Glu-Ala-Asp) box polypepti... 41	0.034	A
contig09398	gi|109130171|ref|XP_001086342.1| PREDICTED: hypothetical protein... 41	0.034	A
contig10593	gi|194669470|ref|XP_615781.4| PREDICTED: similar to jumonji doma... 41	0.034	L
contig12468	gi|48139121|ref|XP_393443.1| PREDICTED: similar to mitochondrial... 41	0.034	J
contig00242	gi|195457344|ref|XP_002075533.1| GK18547 [*Drosophila willistoni*]... 42	0.032	L
contig10049	gi|156370098|ref|XP_001628309.1| predicted protein [*Nematostella*... 42	0.021	A
contig02922	gi|16197971|gb|AAL13756.1| LD23445p [*Drosophila melanogaster*] 42	0.020	A
contig07769	gi|195586770|ref|XP_002083143.1| GD13507 [*Drosophila simulans*] >... 42	0.020	A
contig10091	gi|195128859|ref|XP_002008877.1| GI11570 [*Drosophila mojavensis*]... 42	0.020	A
contig08528	gi|148708536|gb|EDL40483.1| activating signal cointegrator 1 com... 42	0.016	K
contig11141	gi|157126535|ref|XP_001660916.1| sterol regulatory element-bindi... 42	0.015	K
contig05517	gi|115458062|ref|NP_001052631.1| Os04g0388600 [*Oryza sativa* (*jap*... 43	0.009	A
contig09216	gi|195572497|ref|XP_002104232.1| GD18561 [*Drosophila simulans*] >... 43	0.009	J
contig03842	gi|156553460|ref|XP_001604505.1| PREDICTED: similar to translati... 43	0.007	J
contig05252	gi|31745178|ref|NP_853634.1| SWI/SNF-related matrix-associated a... 43	0.007	BK
contig05919	gi|50409953|ref|XP_456921.1| hypothetical protein DEHA0A14036g [... 43	0.007	J

Contig	BLAST result	e value	Class
contig12218	gi|195539327|ref|NP_001124190.1| argonaute 1 [*Strongylocentrotus*... 43	0.007	J
contig02715	gi|72127019|ref|XP_792641.1| PREDICTED: similar to RNA pseudouri... 44	0.005	A
contig05850	gi|145611424|ref|XP_368851.2| hypothetical protein MGG_00393 [*Ma*... 44	0.005	B
contig06764	gi|91093050|ref|XP_967369.1| PREDICTED: similar to RpI12 CG13418... 44	0.005	K
contig00263	gi|145253539|ref|XP_001398282.1| hypothetical protein An17g00530... 33	0.004	K
contig00835	gi|51090781|dbj|BAD35259.1| unknown protein [*Oryza sativa Japoni*... 44	0.004	A
contig04049	gi|193676340|ref|XP_001948540.1| PREDICTED: similar to DNA topoi... 44	0.004	B
contig05302	gi|195433847|ref|XP_002064918.1| GK15187 [*Drosophila willistoni*]... 44	0.004	B
contig06002	gi|157116527|ref|XP_001658535.1| ribosomal pseudouridine synthas... 44	0.004	A
contig10330	gi|167517733|ref|XP_001743207.1| predicted protein [*Monosiga bre*... 44	0.004	J
contig10698	gi|156547804|ref|XP_001606248.1| PREDICTED: hypothetical protein... 44	0.004	K
contig04714	gi|170033323|ref|XP_001844527.1| far upstream binding protein [*C*... 45	0.003	A
contig07710	gi|67084083|gb|AAY66976.1| possible mitochondrial ribosomal prot... 44	0.003	J
contig02630	gi|156545235|ref|XP_001604531.1| PREDICTED: similar to replicati... 45	0.002	L
contig03506	gi|195129479|ref|XP_002009183.1| GI11400 [*Drosophila mojavensis*]... 45	0.002	J
contig06339	gi|154684978|ref|YP_001420139.1| YdcI [*Bacillus amyloliquefacien*... 45	0.002	K
contig07177	gi|115916105|ref|XP_781769.2| PREDICTED: similar to KIAA1218 pro... 45	0.002	B
contig07409	gi|196010159|ref|XP_002114944.1| hypothetical protein TRIADDRAFT... 45	0.002	J
contig07448	gi|194679733|ref|XP_001788248.1| PREDICTED: similar to Pre-mRNA ... 45	0.002	A
contig08253	gi|195032827|ref|XP_001988569.1| GH11234 [*Drosophila grimshawi*] ... 45	0.002	A
contig10041	gi|115758159|ref|XP_793245.2| PREDICTED: similar to KIAA1404 pro... 45	0.002	L
contig00109	gi|194222651|ref|XP_001497265.2| PREDICTED: eIF4GI protein [*Equu*... 48	0.001	J
contig01552	gi|126324882|ref|XP_001379147.1| PREDICTED: similar to Mitochond... 45	0.001	J
contig03887	gi|50513542|pdb|1SGL|A Chain A, The Three-Dimensional Structure ... 45	0.001	A
contig05574	gi|149424585|ref|XP_001520526.1| PREDICTED: similar to MGC21874 ... 46	0.001	B
contig10650	gi|47212288|emb|CAF92859.1| unnamed protein product [*Tetraodon n*... 46	0.001	A
contig11149	gi|119574612|gb|EAW54227.1| AT rich interactive domain 5B (MRF1-... 46	0.001	K
contig00404	gi|47208448|emb|CAF93102.1| unnamed protein product [*Tetraodon n*... 47	8E-04	J
contig01876	gi|110748834|ref|XP_623721.2| PREDICTED: similar to mitochondria... 46	8E-04	J
contig09496	gi|72006586|ref|XP_782916.1| PREDICTED: similar to SET domain an... 46	8E-04	BK
contig12768	gi|196236958|ref|ZP_03135749.1| conserved hypothetical protein [... 49	7E-04	K
contig06572	gi|195048072|ref|XP_001992465.1| GH24190 [*Drosophila grimshawi*] ... 47	6E-04	J
contig08332	gi|195503187|ref|XP_002098546.1| GE23879 [*Drosophila yakuba*] >gn... 47	6E-04	A
contig09868	gi|110759186|ref|XP_396401.3| PREDICTED: similar to GLE1 RNA exp... 47	5E-04	A
contig02142	gi|91083973|ref|XP_975149.1| PREDICTED: similar to splicing fact... 47	4E-04	A
contig05266	gi|115627983|ref|XP_001176368.1| PREDICTED: hypothetical protein... 47	4E-04	A
contig09300	gi|50740707|ref|XP_419536.1| PREDICTED: similar to histone methy... 47	4E-04	B
contig06370	gi|115927400|ref|XP_782196.2| PREDICTED: similar to putative RNA... 49	3E-04	A
contig04441	gi|157105173|ref|XP_001648750.1| S1 RNA binding domain protein, ... 49	2E-04	K
contig05205	gi|195121564|ref|XP_002005290.1| GI20404 [*Drosophila mojavensis*]... 48	2E-04	L
contig08027	gi|112983080|ref|NP_001037670.1| Rsf1 [Bombyx mori] >gnl|BL_ORD_... 48	2E-04	A
contig10786	gi|115965534|ref|XP_001194862.1| PREDICTED: similar to rapamycin... 48	2E-04	L
contig05847	gi|156543392|ref|XP_001600067.1| PREDICTED: similar to ATP-depen... 49	1E-04	A
contig09977	gi|37362208|gb|AAQ91232.1| origin recognition complex, subunit 3... 49	1E-04	L
contig09444	gi|195444815|ref|XP_002070042.1| GK11236 [*Drosophila willistoni*]... 50	7E-05	L
contig00270	gi|91078526|ref|XP_970233.1| PREDICTED: similar to CRSP complex ... 50	6E-05	K
contig01533	gi|56159959|gb|AAV80791.1| putative secreted salivary gland pept... 50	6E-05	A
contig02446	gi|114052735|ref|NP_001040494.1| U2 small nuclear ribonucleoprot... 50	6E-05	A
contig12255	gi|56283929|ref|NP_523475.3| Salivary gland secretion 1 CG3047-P... 53	5E-05	A
contig10347	gi|157761629|ref|XP_001673157.1| Hypothetical protein CBG01314 [... 50	4E-05	J
contig10360	gi|195977039|gb|ACG63590.1| mediator of RNA polymerase II transc... 50	4E-05	K

Contig	BLAST result	e value	Class
contig05024	gi|147901564|ref|NP_001081653.1| ligase I [*Xenopus laevis*] >gnl|... 51	3E-05	L
contig07389	gi|156368841|ref|XP_001627900.1| predicted protein [*Nematostella*... 51	3E-05	K
contig05011	gi|154721379|gb|ABS84820.1| polyprotein [Honey bee slow paralysi... 52	2E-05	J
contig05178	gi|110757968|ref|XP_394707.3| PREDICTED: similar to M-phase phos... 52	2E-05	A
contig07634	gi|194381256|dbj|BAG58582.1| unnamed protein product [*Homo sapiens*] 52	2E-05	K
contig03134	gi|158298514|ref|XP_318687.4| AGAP009650-PA [*Anopheles gambiae* s... 52	1E-05	K
contig04017	gi|112983034|ref|NP_001037070.1| putative H3K9 methyltransferase... 52	1E-05	B
contig06337	gi|170047547|ref|XP_001851279.1| quaking protein A [*Culex quinqu*... 52	1E-05	A
contig03734	gi|73981226|ref|XP_540261.2| PREDICTED: similar to WD-repeat pro... 53	9E-06	A
contig06534	gi|147899310|ref|NP_001085393.1| MGC79017 protein [*Xenopus laevi*... 53	9E-06	L
contig01228	gi|74136739|ref|NP_001028175.1| basic transcription factor 3 [*Ci*... 55	8E-06	K
contig03455	gi|197632259|gb|ACH70853.1| eukaryotic translation initiation fa... 53	7E-06	J
contig04303	gi|156408277|ref|XP_001641783.1| predicted protein [*Nematostella*... 53	7E-06	A
contig04979	gi|170091308|ref|XP_001876876.1| predicted protein [*Laccaria bic*... 53	7E-06	J
contig06877	gi|169158574|emb|CAQ15578.1| novel protein similar to *H.sapiens* ... 54	5E-06	J
contig06964	gi|198151133|gb|EAL30213.2| GA15241 [*Drosophila pseudoobscura* ps... 54	5E-06	J
contig05988	gi|157128864|ref|XP_001655231.1| DNA repair protein xp-e [*Aedes* ... 54	4E-06	L
contig09396	gi|196007374|ref|XP_002113553.1| hypothetical protein TRIADDRAFT... 54	4E-06	K
contig04464	gi|41054625|ref|NP_955870.1| splicing factor, arginine/serine-ri... 54	3E-06	A
contig07230	gi|170040111|ref|XP_001847855.1| elongation factor G 1, mitochon... 54	3E-06	J
contig07745	gi|195401220|ref|XP_002059212.1| GJ16153 [*Drosophila virilis*] >g... 54	3E-06	K
contig10652	gi|187469812|gb|AAI67080.1| Unknown (protein for IMAGE:8363520) ... 54	3E-06	L
contig01465	gi|196476795|gb|ACG76261.1| small nuclear ribonucleoprotein G [A... 55	2E-06	A
contig02953	gi|170084049|ref|XP_001873248.1| predicted protein [*Laccaria bic*... 55	2E-06	J
contig11631	gi|125583414|gb|EAZ24345.1| hypothetical protein OsJ_007828 [*Ory*... 55	2E-06	B
contig11635	gi|82249026|sp|Q9YGX5|CPEB1_BRARE Cytoplasmic polyadenylation el... 55	2E-06	J
contig01711	gi|193678853|ref|XP_001945895.1| PREDICTED: similar to Eukaryoti... 56	1E-06	J
contig02143	gi|170070381|ref|XP_001869559.1| histone-lysine n-methyltransfer... 56	1E-06	BK
contig05304	gi|148234716|ref|NP_001080984.1| flap endonuclease 1 [*Xenopus la*... 52	1E-06	L
contig08532	gi|195552807|ref|XP_002076543.1| GD17572 [*Drosophila simulans*] >... 56	8E-07	J
contig12761	gi|115968078|ref|XP_001189793.1| PREDICTED: similar to dpy-30-li... 56	8E-07	K
contig09466	gi|126334326|ref|XP_001377008.1| PREDICTED: hypothetical protein... 46	7E-07	L
contig01879	gi|194223833|ref|XP_001492097.2| PREDICTED: splicing factor, arg... 57	6E-07	A
contig07782	gi|47210503|emb|CAF90142.1| unnamed protein product [*Tetraodon n*... 57	6E-07	J
contig00143	gi|195972757|dbj|BAG68513.1| histone 4 [*Drosophila takahashii*] 57	5E-07	B
contig10392	gi|195032188|ref|XP_001988454.1| GH11175 [*Drosophila grimshawi*] ... 57	5E-07	J
contig07924	gi|195112849|ref|XP_002000984.1| GI22233 [*Drosophila mojavensis*]... 57	4E-07	A
contig02602	gi|195398566|ref|XP_002057892.1| GJ17848 [*Drosophila virilis*] >g... 60	3E-07	K
contig01115	gi|58396614|ref|XP_322026.2| AGAP001135-PA [*Anopheles gambiae* st... 41	2E-07	L
contig01659	gi|189536204|ref|XP_001335519.2| PREDICTED: similar to mKIAA0191... 58	2E-07	A
contig04995	gi|156377136|ref|XP_001630713.1| predicted protein [*Nematostella*... 59	1E-07	J
contig11061	gi|125985901|ref|XP_001356714.1| GA15004-PA [*Drosophila pseudoob*... 59	1E-07	A
contig02553	gi|156551437|ref|XP_001604411.1| PREDICTED: similar to fetal glo... 59	9E-08	K
contig09577	gi|118404512|ref|NP_001072914.1| mitochondrial ribosomal protein... 59	9E-08	J
contig06417	gi|156540682|ref|XP_001599742.1| PREDICTED: similar to conserved... 61	8E-08	J
contig01064	gi|114561793|ref|YP_749306.1| DEAD/DEAH box helicase domain-cont... 60	7E-08	A
contig06775	gi|66509028|ref|XP_396048.2| PREDICTED: similar to damage specif... 60	7E-08	L
contig10430	gi|194745508|ref|XP_001955230.1| GF16340 [*Drosophila ananassae*] ... 60	7E-08	A
contig10781	gi|189238915|ref|XP_969200.2| PREDICTED: similar to DNA polymera... 60	6E-08	L
contig06284	gi|50744652|ref|XP_419816.1| PREDICTED: similar to Activating si... 48	5E-08	A
contig09079	gi|194213999|ref|XP_001490279.2| PREDICTED: SWI/SNF related, mat... 60	5E-08	BK

Contig	BLAST result	e value	Class
contig07676	gi|114051417|ref|NP_001040314.1| DNA-damage inducible protein [B... 60	4E-08	L
contig10509	gi|126304454|ref|XP_001382178.1| PREDICTED: similar to homolog o... 60	4E-08	L
contig05940	gi|195479102|ref|XP_002100763.1| GE17244 [*Drosophila yakuba*] >gn... 61	3E-08	K
contig08405	gi|189236986|ref|XP_001810629.1| PREDICTED: similar to General t... 61	3E-08	K
contig12471	gi|57525409|ref|NP_001006243.1| minichromosome maintenance compl... 61	3E-08	L
contig04650	gi|156547291|ref|XP_001606432.1| PREDICTED: similar to cysteinyl... 62	2E-08	J
contig05054	gi|149427386|ref|XP_001521089.1| PREDICTED: similar to cap bindi... 62	2E-08	A
contig05706	gi|156554094|ref|XP_001601319.1| PREDICTED: similar to tata-bind... 62	2E-08	K
contig10755	gi|148231815|ref|NP_001082292.1| ARC105 protein [*Xenopus laevis*]... 62	2E-08	K
contig03296	gi|194332576|ref|NP_001123777.1| hypothetical protein LOC1001705... 62	1E-08	L
contig07789	gi|156361828|ref|XP_001625486.1| predicted protein [*Nematostella*... 62	1E-08	A
contig07802	gi|158300342|ref|XP_320293.4| AGAP012249-PA [Anopheles gambiae s... 62	1E-08	J
contig11178	gi|114052779|ref|NP_001040341.1| mitochondrial ribosomal protein... 54	1E-08	J
contig00336	gi|168000575|ref|XP_001752991.1| argonaute family member [*Physco*... 63	8E-09	J
contig01434	gi|112983360|ref|NP_001037644.1| polyadenylate binding protein 2... 65	8E-09	A
contig02240	gi|115727635|ref|XP_789513.2| PREDICTED: similar to MGC82596 pro... 63	8E-09	A
contig03867	gi|156406660|ref|XP_001641163.1| predicted protein [*Nematostella*... 51	8E-09	J
contig02781	gi|189234753|ref|XP_974490.2| PREDICTED: similar to STE20-like k... 63	6E-09	A
contig07124	gi|118100342|ref|XP_001235387.1| PREDICTED: hypothetical protein... 63	6E-09	A
contig03082	gi|195035783|ref|XP_001989351.1| GH11680 [*Drosophila grimshawi*] ... 64	5E-09	J
contig07329	gi|195586452|ref|XP_002082988.1| GD24941 [*Drosophila simulans*] >... 64	5E-09	K
contig13058	gi|156399409|ref|XP_001638494.1| predicted protein [*Nematostella*... 57	5E-09	J
contig09302	gi|125818968|ref|XP_695864.2| PREDICTED: similar to SWI/SNF-rela... 64	4E-09	B
contig10283	gi|118101846|ref|XP_417852.2| PREDICTED: hypothetical protein [*G*... 64	4E-09	J
contig07511	gi|189241033|ref|XP_971712.2| PREDICTED: similar to AGAP012195-P... 65	2E-09	J
contig00575	gi|170581661|ref|XP_001895780.1| transcription elongation factor... 67	1E-09	K
contig02904	gi|56269784|gb|AAH86970.1| Top2a protein [*Danio rerio*] >gnl|BL_O... 66	1E-09	B
contig02948	gi|194224218|ref|XP_001497231.2| PREDICTED: similar to nucleolar... 65	1E-09	AJ
contig05461	gi|189235069|ref|XP_974729.2| PREDICTED: similar to nocturnin CG... 65	1E-09	K
contig08377	gi|66534191|ref|XP_624580.1| PREDICTED: similar to Chromatin ass... 58	1E-09	B
contig08722	gi|170051312|ref|XP_001861707.1| translation machinery-associate... 66	1E-09	J
contig13178	gi|189353843|ref|YP_001949470.1| hypothetical protein BMULJ_0509... 66	1E-09	K
contig03723	gi|195583628|ref|XP_002081619.1| GD25618 [*Drosophila simulans*] >... 66	8E-10	L
contig07492	gi|118100644|ref|XP_417395.2| PREDICTED: similar to KIAA1404 pro... 66	8E-10	L
contig00640	gi|194222435|ref|XP_001500218.2| PREDICTED: splicing factor 3b, ... 67	6E-10	A
contig01565	gi|48101238|ref|XP_392654.1| PREDICTED: similar to mitochondrial... 67	6E-10	J
contig05405	gi|158286351|ref|XP_308698.4| AGAP007065-PA [*Anopheles gambiae* s... 67	6E-10	K
contig02102	gi|157339020|emb|CAO42371.1| unnamed protein product [*Vitis vini*... 67	4E-10	A
contig06393	gi|62078847|ref|NP_001014079.1| tRNA splicing endonuclease 2 hom... 67	4E-10	J
contig02702	gi|61651808|ref|NP_001013344.1| hypothetical protein LOC503748 [... 68	3E-10	L
contig07722	gi|126297559|ref|XP_001362254.1| PREDICTED: similar to Polymeras... 68	3E-10	K
contig09861	gi|47210231|emb|CAF95218.1| unnamed protein product [*Tetraodon n*... 64	3E-10	J
contig00813	gi|115663128|ref|XP_795489.2| PREDICTED: similar to THO complex ... 68	2E-10	K
contig04890	gi|126321954|ref|XP_001366911.1| PREDICTED: similar to E2F-3 tra... 48	2E-10	K
contig05203	gi|170043518|ref|XP_001849432.1| puff-specific protein Bx42 [*Cul*... 69	2E-10	AB
contig09508	gi|91087083|ref|XP_974948.1| PREDICTED: similar to AGAP008405-PA... 68	2E-10	J
contig12019	gi|188593364|emb|CAO78751.1| Oikopleura cytoplasmic RNAse III en... 70	2E-10	A
contig10724	gi|196006696|ref|XP_002113214.1| hypothetical protein TRIADDRAFT... 69	1E-10	A
contig04250	gi|156550801|ref|XP_001600558.1| PREDICTED: similar to FTP3 [*Nas*... 69	9E-11	A
contig07470	gi|66557991|ref|XP_625041.1| PREDICTED: similar to CG11265-PA, i... 70	7E-11	L
contig09166	gi|114683663|ref|XP_525432.2| PREDICTED: hypothetical protein [*P*... 70	7E-11	K

Contig	BLAST result	e value	Class
contig13342	gi|194385764|dbj|BAG65257.1| unnamed protein product [*Homo sapiens*] 70	7E-11	A
contig00489	gi|187023422|emb|CAP37383.1| C. briggsae CBR-TOP-1 protein [*Caen*... 70	5E-11	L
contig10645	gi|163868323|ref|YP_001609532.1| DNA polymerase III subunit delt... 70	5E-11	L
contig08873	gi|58383228|ref|XP_312466.2| AGAP002472-PA [Anopheles gambiae st... 70	4E-11	L
contig12936	gi|197127145|gb|ACH43643.1| putative ribosomal protein S11 [*Taen*... 70	4E-11	J
contig01555	gi|170571964|ref|XP_001891933.1| piwi domain containing protein ... 71	3E-11	J
contig03855	gi|156545501|ref|XP_001607052.1| PREDICTED: similar to cleavage ... 71	3E-11	A
contig02888	gi|189233619|ref|XP_970422.2| PREDICTED: similar to arylhydrocar... 71	2E-11	K
contig08901	gi|110762326|ref|XP_001120377.1| PREDICTED: similar to CG8232-PA... 71	2E-11	L
contig03389	gi|66505576|ref|XP_393347.2| PREDICTED: similar to CG3527-PA [*Ap*... 72	1E-11	J
contig03504	gi|189467630|ref|ZP_03016415.1| hypothetical protein BACINT_0402... 72	1E-11	A
contig04293	gi|158290481|ref|XP_312084.4| AGAP002829-PA [*Anopheles gambiae* s... 72	1E-11	A
contig06499	gi|193704612|ref|XP_001945957.1| PREDICTED: similar to DNA repli... 72	1E-11	L
contig06840	gi|156537721|ref|XP_001608262.1| PREDICTED: similar to GA15333-P... 72	1E-11	J
contig11474	gi|110756942|ref|XP_395357.3| PREDICTED: similar to Rox8 CG5422-... 72	1E-11	AJ
contig13289	gi|77736399|ref|NP_001029899.1| X-box binding protein 1 [*Bos tau*... 73	1E-11	K
contig00439	gi|157674639|gb|ABV60408.1| RNA polymerase II transcriptional co... 52	7E-12	K
contig05288	gi|193610701|ref|XP_001951408.1| PREDICTED: similar to U3 small ... 73	6E-12	A
contig07141	gi|157104333|ref|XP_001648358.1| mitochondrial 28S ribosomal pro... 73	6E-12	J
contig11421	gi|195470256|ref|XP_002087424.1| GE16791 [*Drosophila yakuba*] >gn... 73	6E-12	B
contig02115	gi|193683845|ref|XP_001950620.1| PREDICTED: similar to CG17838 C... 74	5E-12	A
contig04630	gi|165972435|ref|NP_001107069.1| eukaryotic translation initiati... 74	5E-12	J
contig05536	gi|193718435|ref|XP_001949512.1| PREDICTED: similar to conserved... 74	5E-12	J
contig07189	gi|195436461|ref|XP_002066186.1| GK22226 [*Drosophila willistoni*]... 74	5E-12	A
contig04542	gi|157127307|ref|XP_001654915.1| Srb7p: RNA polymerase II holoen... 74	4E-12	K
contig02195	gi|48094369|ref|XP_392115.1| PREDICTED: similar to eIF6 CG17611-... 74	3E-12	J
contig04031	gi|170038522|ref|XP_001847098.1| mitochondrial 28S ribosomal pro... 74	3E-12	J
contig12959	gi|189238875|ref|XP_973671.2| PREDICTED: similar to DNA replicat... 74	3E-12	L
contig00994	gi|156547287|ref|XP_001606343.1| PREDICTED: similar to RE29053p ... 75	2E-12	J
contig01282	gi|110764913|ref|XP_624290.2| PREDICTED: similar to eukaryotic t... 75	2E-12	J
contig05441	gi|156548556|ref|XP_001605611.1| PREDICTED: similar to eukaryoti... 75	2E-12	J
contig00337	gi|47211075|emb|CAF89690.1| unnamed protein product [*Tetraodon n*... 78	1E-12	J
contig03634	gi|189517411|ref|XP_001922483.1| PREDICTED: similar to MGC84354 ... 76	1E-12	A
contig06960	gi|126305772|ref|XP_001375367.1| PREDICTED: similar to NOL1/NOP2... 75	1E-12	J
contig09037	gi|170050832|ref|XP_001861489.1| tRNA methyltransferase [*Culex q*... 75	1E-12	J
contig10244	gi|170031954|ref|XP_001843848.1| U4/U6 small nuclear ribonucleop... 76	7E-13	A
contig06580	gi|34785805|gb|AAH57529.1| Supt5h protein [*Danio rerio*] 77	6E-13	K
contig07992	gi|73981244|ref|XP_540263.2| PREDICTED: similar to Tryptophanyl-... 77	6E-13	J
contig03895	gi|15891751|ref|NP_357423.1| ABC transporter, substrate binding ... 77	3E-13	K
contig05364	gi|71021341|ref|XP_760901.1| hypothetical protein UM04754.1 [*Ust*... 77	3E-13	A
contig00361	gi|82249026|sp|Q9YGX5|CPEB1_BRARE Cytoplasmic polyadenylation el... 79	2E-13	J
contig01574	gi|170028476|ref|XP_001842121.1| conserved hypothetical protein ... 78	2E-13	A
contig06467	gi|156389050|ref|XP_001634805.1| predicted protein [*Nematostella*... 78	2E-13	L
contig11590	gi|149287022|gb|ABR23410.1| p8 nuclear protein [*Ornithodoros par*... 80	2E-13	K
contig11032	gi|156555684|ref|XP_001602582.1| PREDICTED: hypothetical protein... 79	1E-13	A
contig11219	gi|170035603|ref|XP_001845658.1| gar2 [*Culex quinquefasciatus*] >... 79	1E-13	J
contig12333	gi|158300914|ref|XP_320713.3| AGAP011802-PA [*Anopheles gambiae* s... 79	9E-14	J
contig06202	gi|156552643|ref|XP_001603250.1| PREDICTED: similar to CG6049-PB... 80	7E-14	K
contig09288	gi|195375100|ref|XP_002046341.1| GJ12560 [*Drosophila virilis*] >g... 80	7E-14	J
contig05577	gi|47221361|emb|CAF97279.1| unnamed protein product [*Tetraodon n*... 70	6E-14	J
contig02618	gi|195570991|ref|XP_002103487.1| GD18948 [*Drosophila simulans*] >... 80	5E-14	K

Contig	BLAST result	e value	Class
contig05166	gi|125977188|ref|XP_001352627.1| GA13435-PA [*Drosophila pseudoob*... 80	5E-14	J
contig12287	gi|118097495|ref|XP_414612.2| PREDICTED: similar to CCR4-NOT tra... 80	5E-14	K
contig09047	gi|46129443|ref|XP_389073.1| hypothetical protein FG08897.1 [*Gib*... 80	4E-14	A
contig07299	gi|194440948|gb|ACF70735.1| eukaryotic translation elongation fa... 81	3E-14	J
contig12004	gi|193603617|ref|XP_001950573.1| PREDICTED: similar to eukaryoti... 84	3E-14	J
contig01996	gi|149286958|gb|ABR23378.1| 60S ribosomal protein L29 [*Ornithodo*... 81	2E-14	J
contig05028	gi|172051058|gb|ACB70304.1| histone 1 [*Ornithodoros coriaceus*] 82	2E-14	B
contig05698	gi|195383944|ref|XP_002050685.1| GJ20075 [*Drosophila virilis*] >g... 82	2E-14	J
contig08617	gi|115687312|ref|XP_793463.2| PREDICTED: similar to Lysyl-tRNA s... 81	2E-14	J
contig09083	gi|195425857|ref|XP_002061179.1| GK10341 [*Drosophila willistoni*]... 82	2E-14	J
contig09914	gi|91082415|ref|XP_970120.1| PREDICTED: similar to tRNA-dihydrou... 82	2E-14	J
contig11563	gi|172051058|gb|ACB70304.1| histone 1 [*Ornithodoros coriaceus*] 84	2E-14	B
contig02713	gi|110770107|ref|XP_001123058.1| PREDICTED: similar to Rbp1-like... 83	1E-14	A
contig04498	gi|195438186|ref|XP_002067018.1| GK24781 [*Drosophila willistoni*]... 82	1E-14	A
contig07339	gi|122890852|emb|CAM12938.1| poly(A) polymerase gamma [*Danio rerio*] 82	1E-14	A
contig12018	gi|194500479|gb|ACF75502.1| histone H2B [*Philodina roseola*] 82	1E-14	B
contig03464	gi|193634355|ref|XP_001949691.1| PREDICTED: similar to eukaryoti... 83	8E-15	J
contig12593	gi|115618688|ref|XP_793195.2| PREDICTED: hypothetical protein [S... 83	6E-15	K
contig00254	gi|156555067|ref|XP_001604099.1| PREDICTED: similar to smooth [N... 84	5E-15	A
contig08266	gi|170035806|ref|XP_001845758.1| ATP-dependent RNA helicase p62 ... 84	5E-15	A
contig01672	gi|125980520|ref|XP_001354284.1| GA11463-PA [*Drosophila pseudoob*... 84	4E-15	J
contig02633	gi|195447052|ref|XP_002071043.1| GK25580 [*Drosophila willistoni*]... 84	3E-15	J
contig05163	gi|4691352|emb|CAB41634.1| iron regulatory protein 1-like protei... 84	3E-15	AJ
contig12034	gi|41055136|ref|NP_957497.1| HIV TAT specific factor 1 [*Danio re*... 84	3E-15	K
contig09958	gi|118100214|ref|XP_423183.2| PREDICTED: similar to putative chr... 85	2E-15	A
contig03575	gi|114153222|gb|ABI52777.1| conserved arthropod protein [*Argas m*... 87	1E-15	K
contig06654	gi|170590770|ref|XP_001900144.1| Brix domain containing protein ... 86	1E-15	A
contig09238	gi|45361635|ref|NP_989393.1| hypothetical protein LOC395030 [*Xen*... 86	9E-16	L
contig09401	gi|58332582|ref|NP_001011365.1| cysteinyl-tRNA synthetase [*Xenop*... 86	9E-16	J
contig04141	gi|156358627|ref|XP_001624618.1| predicted protein [*Nematostella*... 86	7E-16	A
contig10297	gi|115677476|ref|XP_001203441.1| PREDICTED: similar to DEAH (Asp... 86	7E-16	A
contig09185	gi|169260671|gb|ACA52061.1| vasa [*Anopheles gambiae*] 87	6E-16	A
contig03872	gi|195114712|ref|XP_002001911.1| GI17096 [*Drosophila mojavensis*]... 87	5E-16	J
contig00508	gi|195442109|ref|XP_002068802.1| GK17973 [*Drosophila willistoni*]... 87	4E-16	A
contig04075	gi|194385024|dbj|BAG60918.1| unnamed protein product [*Homo sapiens*] 87	4E-16	JK
contig06701	gi|193598839|ref|XP_001949841.1| PREDICTED: similar to ATP-depen... 87	4E-16	A
contig06273	gi|114052923|ref|NP_001040037.1| asparaginyl-tRNA synthetase [*Bo*... 88	2E-16	J
contig06482	gi|148224586|ref|NP_001085709.1| MGC80562 protein [*Xenopus laevi*... 76	2E-16	A
contig09227	gi|193617855|ref|XP_001949062.1| PREDICTED: similar to smooth CG... 89	1E-16	A
contig03531	gi|148223529|ref|NP_001086279.1| MGC84435 protein [*Xenopus laevi*... 89	8E-17	B
contig13282	gi|197210363|gb|ACH48189.1| alternative splicing factor SRp20/9G... 93	6E-17	A
contig03621	gi|2352418|gb|AAC05332.1| extra sex combs [*Schistocerca americana*] 62	5E-17	K
contig04437	gi|149633871|ref|XP_001512293.1| PREDICTED: hypothetical protein... 90	5E-17	A
contig08331	gi|193627300|ref|XP_001946602.1| PREDICTED: similar to twist [*Ac*... 90	5E-17	K
contig10867	gi|156549811|ref|XP_001606554.1| PREDICTED: hypothetical protein... 90	5E-17	A
contig07373	gi|66516301|ref|XP_623215.1| PREDICTED: similar to egalitarian C... 91	4E-17	L
contig02322	gi|156545066|ref|XP_001601066.1| PREDICTED: similar to mediator ... 92	2E-17	K
contig04035	gi|158301455|ref|XP_321146.4| AGAP001917-PA [*Anopheles gambiae* s... 92	2E-17	BK
contig05127	gi|196006025|ref|XP_002112879.1| hypothetical protein TRIADDRAFT... 91	2E-17	J
contig08119	gi|194381198|dbj|BAG64167.1| unnamed protein product [*Homo sapiens*] 92	2E-17	A
contig09623	gi|47214330|emb|CAG00839.1| unnamed protein product [*Tetraodon n*... 92	2E-17	K

Contig	BLAST result	e value	Class
contig00689	gi|149286900|gb|ABR23349.1| 40S ribosomal protein S28 [*Ornithodo*... 92	1E-17	J
contig03566	gi|195398847|ref|XP_002058032.1| GJ15858 [*Drosophila virilis*] >g... 92	1E-17	J
contig03761	gi|156398337|ref|XP_001638145.1| predicted protein [*Nematostella*... 94	9E-18	A
contig06432	gi|50761009|ref|XP_425893.1| PREDICTED: similar to ATF family tr... 93	8E-18	K
contig01759	gi|66519677|ref|XP_393827.2| PREDICTED: similar to Big brother C... 93	6E-18	K
contig04487	gi|74004026|ref|XP_545326.2| PREDICTED: similar to Phenylalanyl-... 93	6E-18	J
contig06505	gi|91088779|ref|XP_967442.1| PREDICTED: similar to SD10981p [*Tri*... 93	6E-18	J
contig07151	gi|195447876|ref|XP_002071410.1| GK25783 [*Drosophila willistoni*]... 93	6E-18	J
contig03282	gi|118343958|ref|NP_001071803.1| transcription factor protein [*C*... 57	5E-18	K
contig11682	gi|194225519|ref|XP_001495451.2| PREDICTED: similar to Nuclear c... 95	5E-18	A
contig02042	gi|115938145|ref|XP_786336.2| PREDICTED: hypothetical protein [*S*... 94	3E-18	A
contig02045	gi|154091255|gb|ABS57437.1| ribosomal protein P2 [*Heliconius mel*... 95	3E-18	J
contig06620	gi|115696664|ref|XP_001199006.1| PREDICTED: similar to Eukaryoti... 94	3E-18	J
contig02871	gi|163868316|ref|YP_001609525.1| 30S ribosomal protein S4 [*Barto*... 95	2E-18	J
contig05621	gi|193603584|ref|XP_001948892.1| PREDICTED: similar to DNA polym... 84	2E-18	L
contig11745	gi|115614630|ref|XP_001199821.1| PREDICTED: similar to fetal glo... 95	2E-18	K
contig00807	gi|188529679|gb|ACD62526.1| Vasa short form [*Silurus meridionalis*] 96	9E-19	A
contig03635	gi|149641567|ref|XP_001511623.1| PREDICTED: hypothetical protein... 96	9E-19	J
contig03921	gi|195570494|ref|XP_002103242.1| GD20313 [*Drosophila simulans*] >... 96	9E-19	B
contig04658	gi|167525803|ref|XP_001747236.1| predicted protein [*Monosiga bre*... 96	9E-19	J
contig11018	gi|195445281|ref|XP_002070256.1| GK11133 [*Drosophila willistoni*]... 96	9E-19	J
contig05852	gi|189237999|ref|XP_001812951.1| PREDICTED: similar to Mediator ... 96	7E-19	K
contig12348	gi|115941338|ref|XP_001196755.1| PREDICTED: similar to SAS10 [St... 97	5E-19	B
contig07653	gi|66499810|ref|XP_624522.1| PREDICTED: similar to nudix (nucleo... 97	3E-19	A
contig10376	gi|156537177|ref|XP_001604206.1| PREDICTED: similar to ribosome ... 97	3E-19	J
contig10759	gi|118404292|ref|NP_001072455.1| protein inhibitor of activated ... 75	3E-19	K
contig00238	gi|91090322|ref|XP_972497.1| PREDICTED: similar to AGAP012204-PA... 98	2E-19	A
contig00533	gi|195377858|ref|XP_002047704.1| dead box protein 80 [*Drosophila*... 98	2E-19	A
contig02033	gi|157117813|ref|XP_001653048.1| hypothetical protein AaeL_AAEL0... 98	2E-19	K
contig04340	gi|158298472|ref|XP_318643.3| AGAP009613-PA [*Anopheles gambiae* s... 98	2E-19	J
contig04491	gi|91087281|ref|XP_975549.1| PREDICTED: similar to conserved hyp... 98	2E-19	L
contig06096	gi|194222435|ref|XP_001500218.2| PREDICTED: splicing factor 3b, ... 98	2E-19	A
contig10617	gi|47215698|emb|CAG04782.1| unnamed protein product [*Tetraodon n*... 98	2E-19	KLB
contig13219	gi|156397042|ref|XP_001637701.1| predicted protein [*Nematostella*... 99	2E-19	J
contig01400	gi|193599122|ref|XP_001948282.1| PREDICTED: similar to chromodom... 99	1E-19	K
contig05379	gi|149898916|gb|ABR27968.1| putative elongation factor 1 beta [*T*... 99	1E-19	K
contig05458	gi|157138343|ref|XP_001657254.1| hypothetical protein AaeL_AAEL0... 99	8E-20	K
contig00948	gi|158120678|gb|ABW16870.1| ribosomal protein P0 [*Haemaphysalis* ... 100	7E-20	J
contig06201	gi|193582379|ref|XP_001951043.1| PREDICTED: similar to conserved... 100	6E-20	AR
contig06682	gi|10121857|gb|AAG13401.1|AF285155_1 topoisomerase II alpha-2 [*G*... 100	5E-20	B
contig07732	gi|110765057|ref|XP_397167.3| PREDICTED: similar to pitchoune CG... 100	5E-20	A
contig06809	gi|110763164|ref|XP_001122465.1| PREDICTED: similar to Stem-loop... 100	4E-20	A
contig04267	gi|25027443|ref|NP_737497.1| hypothetical protein CE0887 [*Coryne*... 100	3E-20	K
contig09360	gi|47210118|emb|CAF91684.1| unnamed protein product [*Tetraodon n*... 100	3E-20	K
contig12217	gi|115447849|ref|NP_001047704.1| Os02g0672200 [*Oryza sativa* (*jap*... 74	3E-20	J
contig02463	gi|195381389|ref|XP_002049435.1| GJ21579 [*Drosophila virilis*] >g... 101	2E-20	J
contig03519	gi|156538236|ref|XP_001602268.1| PREDICTED: similar to helicase ... 101	2E-20	L
contig09970	gi|170592815|ref|XP_001901160.1| Yeast mcm [*Brugia malayi*] >gnl|... 101	2E-20	L
contig08633	gi|193702205|ref|XP_001943193.1| PREDICTED: similar to bifunctio... 102	1E-20	J
contig12241	gi|91084417|ref|XP_967827.1| PREDICTED: similar to chromobox-lik... 104	8E-21	B
contig03149	gi|195069805|ref|XP_001997032.1| GH23229 [*Drosophila grimshawi*] ... 102	7E-21	A

Contig	BLAST result	e value	Class
contig08764	gi|193290168|ref|NP_001123270.1| pitchoune [*Nasonia vitripennis*] 103	6E-21	A
contig09517	gi|194039507|ref|XP_001927776.1| PREDICTED: mortality factor 4 l... 103	6E-21	BK
contig04492	gi|189237154|ref|XP_973939.2| PREDICTED: similar to igf2 mRNA bi... 104	3E-21	AR
contig09075	gi|163868076|ref|YP_001609280.1| trigger factor [*Bartonella trib*... 103	3E-21	A
contig02435	gi|156551567|ref|XP_001601734.1| PREDICTED: similar to helicase ... 104	2E-21	K
contig02801	gi|196015281|ref|XP_002117498.1| hypothetical protein TRIADDRAFT... 92	2E-21	A
contig05795	gi|193589588|ref|XP_001948328.1| PREDICTED: similar to eukaryoti... 88	2E-21	J
contig07711	gi|170029969|ref|XP_001842863.1| snrnp sm protein [*Culex quinque*... 104	2E-21	A
contig08083	gi|156385388|ref|XP_001633612.1| predicted protein [*Nematostella*... 104	2E-21	A
contig08903	gi|17986801|ref|NP_539435.1| cold shock protein CSPA [*Brucella m*... 105	2E-21	J
contig13269	gi|67083849|gb|AAY66859.1| ribosomal protein L28 [*Ixodes scapula*... 105	2E-21	J
contig01314	gi|157108382|ref|XP_001650201.1| seryl-tRNA synthetase [*Aedes ae*... 105	1E-21	J
contig03669	gi|195477424|ref|XP_002086336.1| GE22920 [*Drosophila yakuba*] >gn... 106	1E-21	J
contig00297	gi|115908623|ref|XP_783274.2| PREDICTED: similar to MGC83105 pro... 106	5E-22	A
contig01429	gi|153009303|ref|YP_001370518.1| 30S ribosomal protein S5 [*Ochro*... 106	5E-22	J
contig04286	gi|47087413|ref|NP_998606.1| repressor of RNA polymerase III tra... 106	5E-22	K
contig05966	gi|115627171|ref|XP_001188174.1| PREDICTED: similar to Cleavage ... 106	5E-22	A
contig03398	gi|193806389|sp|A6N9N3.1|RL38_ORNPR 60S ribosomal protein L38 >g... 107	4E-22	J
contig00158	gi|114153052|gb|ABI52692.1| ribosomal protein LP1 [*Argas monolak*... 107	3E-22	J
contig03406	gi|110767088|ref|XP_395451.3| PREDICTED: similar to CG40351-PA.3... 107	2E-22	BK
contig05763	gi|47215025|emb|CAG01849.1| unnamed protein product [*Tetraodon n*... 107	2E-22	J
contig07471	gi|115717870|ref|XP_781450.2| PREDICTED: hypothetical protein [*S*... 107	2E-22	J
contig08030	gi|156543507|ref|XP_001602171.1| PREDICTED: similar to CG8144-PK... 107	2E-22	AR
contig12947	gi|91087549|ref|XP_970604.1| PREDICTED: similar to pterin-4a-car... 107	2E-22	K
contig03876	gi|91086147|ref|XP_969343.1| PREDICTED: similar to CG1972 CG1972... 78	1E-22	A
contig04639	gi|194390836|dbj|BAG62177.1| unnamed protein product [*Homo sapiens*] 108	1E-22	J
contig03514	gi|156550205|ref|XP_001601191.1| PREDICTED: similar to conserved... 110	6E-23	KA
contig03796	gi|170053997|ref|XP_001862928.1| mitochondrial ribosomal protein... 110	5E-23	J
contig11079	gi|115744100|ref|XP_001197420.1| PREDICTED: hypothetical protein... 111	5E-23	J
contig08574	gi|156398094|ref|XP_001638024.1| predicted protein [*Nematostella*... 100	4E-23	L
contig12282	gi|75029844|sp|Q4PM64|RS21_IXOSC 40S ribosomal protein S21 >gnl|... 72	4E-23	J
contig06631	gi|157128943|ref|XP_001661560.1| ribosomal RNA small subunit met... 110	3E-23	A
contig02269	gi|91082895|ref|XP_972070.1| PREDICTED: similar to lupus la ribo... 111	2E-23	A
contig10466	gi|126341698|ref|XP_001380514.1| PREDICTED: similar to p300/CBP-... 94	2E-23	BK
contig00708	gi|110761892|ref|XP_391934.3| PREDICTED: similar to CG8636-PA [A... 113	1E-23	J
contig11771	gi|148560314|ref|YP_001259646.1| leucyl-tRNA synthetase [*Brucell*... 112	9E-24	J
contig02872	gi|189235571|ref|XP_001812213.1| PREDICTED: similar to wd-repeat... 112	7E-24	B
contig02031	gi|160871558|ref|ZP_02061690.1| DNA gyrase, B subunit [*Rickettsi*... 113	6E-24	B
contig05684	gi|156383769|ref|XP_001633005.1| predicted protein [*Nematostella*... 113	5E-24	L
contig11337	gi|66559524|ref|XP_393057.2| PREDICTED: similar to Eukaryotic in... 85	4E-24	J
contig03690	gi|156553460|ref|XP_001604505.1| PREDICTED: similar to translati... 114	3E-24	J
contig11586	gi|91086921|ref|XP_971865.1| PREDICTED: similar to Probable mito... 115	3E-24	J
contig00218	gi|149286942|gb|ABR23370.1| 60S ribosomal protein L22 [*Ornithodo*... 115	2E-24	J
contig07272	gi|118790039|ref|XP_317984.3| AGAP004824-PA [*Anopheles gambiae* s... 114	2E-24	J
contig00380	gi|156548880|ref|XP_001606467.1| PREDICTED: similar to conserved... 115	1E-24	L
contig01332	gi|134948671|ref|NP_001077096.1| small nuclear ribonucleoprotein... 115	1E-24	A
contig07569	gi|189238701|ref|XP_968537.2| PREDICTED: similar to Rs1 CG2173-P... 115	1E-24	A
contig11708	gi|158187762|gb|ABW23170.1| ribosomal protein rpl14 [*Arenicola m*... 115	1E-24	J
contig13237	gi|114152964|gb|ABI52648.1| ubiquitin/40S ribosomal protein S27a... 116	1E-24	J
contig04809	gi|156538399|ref|XP_001605688.1| PREDICTED: similar to GA17927-P... 115	9E-25	J
contig03933	gi|170054347|ref|XP_001863087.1| splicing factor u2af large subu... 116	6E-25	A

Contig	BLAST result	e value	Class
contig01646	gi|196000174|ref|XP_002109955.1| hypothetical protein TRIADDRAFT... 116	5E-25	J
contig01929	gi|148298659|ref|NP_001091811.1| mitochondrial ribosomal protein... 116	5E-25	J
contig07995	gi|194670338|ref|XP_877614.3| PREDICTED: similar to transducin-l... 116	5E-25	B
contig08203	gi|126272991|ref|XP_001372133.1| PREDICTED: similar to PD2 prote... 116	5E-25	K
contig04672	gi|194679131|ref|XP_001254710.2| PREDICTED: similar to topoisome... 117	3E-25	B
contig05578	gi|156385037|ref|XP_001633438.1| predicted protein [*Nematostella*... 119	1E-25	J
contig11128	gi|110754971|ref|XP_396833.3| PREDICTED: similar to euchromatic ... 90	1E-25	BK
contig13007	gi|192447401|ref|NP_001122187.1| hypothetical protein LOC563448 ... 118	1E-25	A
contig01436	gi|149287094|gb|ABR23446.1| 40S ribosomal protein S29 [*Ornithodo*... 120	7E-26	J
contig10939	gi|194036047|ref|XP_001928886.1| PREDICTED: similar to Mitochond... 120	7E-26	J
contig13239	gi|15213826|gb|AAK92188.1|AF400216_1 ribosomal protein S19 [*Spod*... 119	6E-26	J
contig09529	gi|162287117|ref|NP_001104751.1| wu:fb18g03 [Danio rerio] >gnl|*B*... 120	4E-26	K
contig02451	gi|119874619|gb|ABM05786.1| dyskerin [*Triops longicaudatus*] 120	3E-26	J
contig03772	gi|62083517|gb|AAX62483.1| mitochondrial ribosomal protein L17 [... 120	3E-26	J
contig10126	gi|166797029|gb|AAI59199.1| Ddx5 protein [*Danio rerio*] 121	2E-26	A
contig11454	gi|198149604|gb|EAL31227.2| GA19033 [*Drosophila pseudoobscura* ps... 121	2E-26	A
contig00324	gi|189239335|ref|XP_973573.2| PREDICTED: similar to pasilla CG16... 122	1E-26	AR
contig02746	gi|163868077|ref|YP_001609281.1| aspartyl/glutamyl-tRNA amidotra... 105	1E-26	J
contig11340	gi|156544606|ref|XP_001602203.1| PREDICTED: similar to EN10 [*Nas*... 122	7E-27	J
contig12939	gi|91083327|ref|XP_974883.1| PREDICTED: similar to 39S ribosomal... 123	5E-27	J
contig01997	gi|193657279|ref|XP_001951507.1| PREDICTED: similar to 67 kDa po... 123	4E-27	JK
contig00421	gi|159145658|gb|ABW90366.1| putative ribosomal protein L35 [*Sipu*... 124	2E-27	J
contig04460	gi|166215964|sp|A2BHJ4.1|CNO6L_DANRE CCR4-NOT transcription comp... 124	2E-27	K
contig07504	gi|170042852|ref|XP_001849125.1| 39S ribosomal protein L45 [*Cule*... 125	1E-27	J
contig04188	gi|66544546|ref|XP_623093.1| PREDICTED: similar to RAD23a homolo... 125	8E-28	L
contig00677	gi|47550803|ref|NP_999933.1| superkiller viralicidic activity 2-... 126	7E-28	A
contig05802	gi|91087799|ref|XP_967345.1| PREDICTED: similar to RH56418p [*Tri*... 126	6E-28	J
contig11627	gi|156544840|ref|XP_001606830.1| PREDICTED: similar to GA13644-P... 126	5E-28	K
contig02691	gi|197129949|gb|ACH46447.1| putative LSM4 homolog U6 small nucle... 127	4E-28	A
contig05067	gi|196007484|ref|XP_002113608.1| hypothetical protein TRIADDRAFT... 127	3E-28	BK
contig11171	gi|91083589|ref|XP_968607.1| PREDICTED: similar to mitochondrial... 115	3E-28	J
contig05781	gi|110759283|ref|XP_397014.3| PREDICTED: similar to absent, smal... 128	2E-28	BK
contig00203	gi|169931071|gb|ACB05775.1| 60S ribosomal protein L37a [*Artemia* ... 129	1E-28	J
contig06069	gi|47218337|emb|CAG04169.1| unnamed protein product [*Tetraodon n*... 129	7E-29	A
contig06299	gi|31127036|gb|AAF26656.2|AF139006_1 RNA polymerase II largest s... 129	7E-29	K
contig01687	gi|156540001|ref|XP_001599059.1| PREDICTED: similar to enhancer ... 129	6E-29	K
contig01748	gi|91085659|ref|XP_971350.1| PREDICTED: similar to Intron-bindin... 129	6E-29	L
contig04857	gi|195998546|ref|XP_002109141.1| hypothetical protein TRIADDRAFT... 130	6E-29	J
contig10077	gi|1351179|sp|P49696|SYV_FUGRU Valyl-tRNA synthetase (Valine--tR... 129	6E-29	J
contig12445	gi|67083895|gb|AAY66882.1| ribosomal protein S25 [*Ixodes scapula*... 129	6E-29	J
contig04440	gi|193683638|ref|XP_001952518.1| PREDICTED: similar to mitochond... 98	5E-29	J
contig04072	gi|195568479|ref|XP_002102243.1| GD19602 [*Drosophila simulans*] >... 130	3E-29	J
contig08726	gi|170039954|ref|XP_001847781.1| conserved hypothetical protein ... 130	3E-29	K
contig06234	gi|153009283|ref|YP_001370498.1| elongation factor G [*Ochrobactr*... 131	2E-29	J
contig00115	gi|156349201|ref|XP_001621959.1| hypothetical protein NEMVEDRAFT... 135	9E-30	A
contig13226	gi|160948300|emb|CAO94751.1| putative ribosomal protein L37 [*Pom*... 133	5E-30	J
contig05504	gi|189234959|ref|XP_973320.2| PREDICTED: similar to nonsense-med... 133	4E-30	A
contig09269	gi|156552599|ref|XP_001601647.1| PREDICTED: similar to 5-3 exori... 134	3E-30	LA
contig00141	gi|197215705|gb|ACH53093.1| CCR4-NOT transcription complex, subu... 134	2E-30	K
contig03982	gi|149636761|ref|XP_001505482.1| PREDICTED: hypothetical protein... 134	2E-30	KL
contig04591	gi|194742728|ref|XP_001953853.1| GF17974 [*Drosophila ananassae*] ... 134	2E-30	J

Contig	BLAST result	e value	Class
contig08305	gi|91093851|ref|XP_970554.1| PREDICTED: similar to pre-mRNA-spli... 134	2E-30	A
contig08512	gi|156543001|ref|XP_001603257.1| PREDICTED: similar to RNA-bindi... 134	2E-30	A
contig01835	gi|149639779|ref|XP_001510132.1| PREDICTED: similar to MGC97718 ... 135	8E-31	A
contig13042	gi|49619137|gb|AAT68153.1| eukaryotic translation initiation fac... 137	5E-31	J
contig02573	gi|197129764|gb|ACH46262.1| putative H3 histone family 3B varian... 137	4E-31	B
contig11750	gi|124487974|gb|ABN12070.1| putative RNA-dependent helicase p68 ... 139	4E-31	A
contig05561	gi|189234539|ref|XP_973056.2| PREDICTED: similar to AGAP001195-P... 94	3E-31	A
contig01453	gi|112982956|ref|NP_001037676.1| splicing factor arginine/serine... 139	2E-31	A
contig12235	gi|83644429|ref|YP_432864.1| hypothetical protein HCH_10036 [*Hah*... 137	2E-31	K
contig07323	gi|115767175|ref|XP_798957.2| PREDICTED: similar to Eukaryotic t... 138	1E-31	J
contig02597	gi|197131003|gb|ACH47049.1| target of rapamycin [*Blattella germa*... 139	9E-32	L
contig07360	gi|195589302|ref|XP_002084391.1| GD14248 [*Drosophila simulans*] >... 139	9E-32	KL
contig07552	gi|168062615|ref|XP_001783274.1| predicted protein [*Physcomitrel*... 139	7E-32	J
contig08491	gi|157103157|ref|XP_001647846.1| elongin b [*Aedes aegypti*] >gnl|... 139	7E-32	K
contig08505	gi|198146545|gb|EDY72729.1| GA29178 [*Drosophila pseudoobscura* ps... 139	7E-32	A
contig10348	gi|149408557|ref|XP_001513499.1| PREDICTED: similar to G elongat... 139	7E-32	J
contig11150	gi|157128852|ref|XP_001655225.1| cytoplasmic polyadenylation ele... 142	7E-32	J
contig07616	gi|110758526|ref|XP_394712.3| PREDICTED: similar to Chip CG3924-... 140	6E-32	K
contig01682	gi|197210381|gb|ACH48198.1| translation initiation factor 5A [*Or*... 141	2E-32	J
contig05282	gi|187936050|gb|ACD37546.1| mitochondrial ribosomal protein s22 ... 141	2E-32	J
contig11608	gi|169153890|emb|CAQ14784.1| novel protein similar to vertebrate... 96	2E-32	BK
contig00335	gi|195359243|ref|XP_002045323.1| GM23254 [*Drosophila sechellia*] ... 145	1E-32	J
contig02049	gi|110758526|ref|XP_394712.3| PREDICTED: similar to Chip CG3924-... 141	1E-32	K
contig05055	gi|91086675|ref|XP_968460.1| PREDICTED: similar to seryl-tRNA sy... 141	1E-32	J
contig05347	gi|51571949|ref|NP_001003978.1| G1 to S phase transition 1 [Ratt... 142	7E-33	J
contig02071	gi|91088509|ref|XP_971592.1| PREDICTED: similar to pre-mRNA-spli... 142	6E-33	A
contig11441	gi|148657070|ref|YP_001277275.1| putative endoribonuclease L-PSP... 143	5E-33	J
contig04975	gi|38490438|emb|CAD57737.1| extradenticle 1 [*Cupiennius salei*] 143	4E-33	K
contig12937	gi|48142237|ref|XP_397314.1| PREDICTED: similar to ribosomal pro... 143	4E-33	J
contig03090	gi|195120149|ref|XP_002004591.1| GI20015 [*Drosophila mojavensis*]... 144	3E-33	J
contig00612	gi|170043665|ref|XP_001849498.1| RNA and export factor binding p... 147	2E-33	A
contig03593	gi|66546305|ref|XP_394723.2| PREDICTED: similar to DEAD (Asp-Glu... 145	1E-33	A
contig12632	gi|73976787|ref|XP_857499.1| PREDICTED: similar to poly(A) bindi... 145	1E-33	AJ
contig12271	gi|195582973|ref|XP_002081300.1| GD10948 [*Drosophila simulans*] >... 145	8E-34	J
contig03838	gi|170047758|ref|XP_001851377.1| DNA topoisomerase 2 [*Culex quin*... 146	6E-34	B
contig10306	gi|195570991|ref|XP_002103487.1| GD18948 [*Drosophila simulans*] >... 146	6E-34	K
contig02425	gi|158256662|dbj|BAF84304.1| unnamed protein product [*Homo sapiens*] 147	3E-34	L
contig06780	gi|193669397|ref|XP_001951491.1| PREDICTED: similar to AGAP00308... 149	7E-35	J
contig01963	gi|156548548|ref|XP_001604962.1| PREDICTED: similar to ENSANGP00... 150	4E-35	K
contig01034	gi|91081091|ref|XP_975490.1| PREDICTED: similar to barrier-to-au... 152	3E-35	BL
contig11477	gi|70909783|emb|CAJ17317.1| ribosomal protein L24e [*Julodis onop*... 150	3E-35	J
contig00227	gi|67084045|gb|AAY66957.1| ribosomal protein L40 [*Ixodes scapula*... 152	2E-35	J
contig13121	gi|90419459|ref|ZP_01227369.1| conserved hypothetical protein [*A*... 151	2E-35	K
contig11835	gi|114145437|ref|NP_001041456.1| transcription factor [*Ciona int*... 151	1E-35	B
contig12873	gi|4689134|gb|AAD27776.1|AF077043_1 60S ribosomal protein L36 [*H*... 153	5E-36	J
contig02584	gi|124504396|gb|AAI28806.1| Phenylalanyl-tRNA synthetase, alpha ... 153	4E-36	J
contig11556	gi|71151998|sp|Q8AWF2|NACA_ORENI Nascent polypeptide-associated ... 158	9E-37	K
contig11522	gi|156539055|ref|XP_001599770.1| PREDICTED: similar to ENSANGP00... 157	7E-37	B
contig02663	gi|51965690|emb|CAG29667.1| translation initiation factor 2 gamm... 156	6E-37	J
contig00386	gi|170041583|ref|XP_001848537.1| ebna2 binding protein P100 [Cul... 158	5E-37	K
contig00686	gi|112983906|ref|NP_001036848.1| En10 protein [*Bombyx mori*] >gnl... 158	5E-37	J

Contig	BLAST result	e value	Class
contig13232	gi|66506394|ref|XP_624700.1| PREDICTED: similar to CG31618-PA [A... 160	3E-37	B
contig05104	gi|194374871|dbj|BAG62550.1| unnamed protein product [*Homo sapiens*] 159	1E-37	L
contig11352	gi|149287044|gb|ABR23421.1| elongation factor 1-beta [*Ornithodor*... 144	8E-38	K
contig02367	gi|157124837|ref|XP_001660547.1| hypothetical protein AaeL_AAEL0... 159	5E-38	J
contig11006	gi|156554609|ref|XP_001604785.1| PREDICTED: similar to sigma DNA... 162	3E-38	L
contig11529	gi|194223075|ref|XP_001498098.2| PREDICTED: similar to Hist1h4c ... 162	3E-38	B
contig11919	gi|156538234|ref|XP_001602190.1| PREDICTED: similar to ENSANGP00... 119	2E-38	K
contig12810	gi|114675653|ref|XP_001170388.1| PREDICTED: deoxyribonuclease II... 119	2E-38	L
contig00230	gi|159145788|gb|ABW90431.1| putative ribosomal protein S27 [*Bare*... 162	1E-38	J
contig03800	gi|148223485|ref|NP_001085851.1| MGC80911 protein [*Xenopus laevi*... 162	8E-39	J
contig04100	gi|115970958|ref|XP_001194184.1| PREDICTED: similar to WD repeat... 164	2E-39	A
contig06768	gi|70909867|emb|CAJ17420.1| ribosomal protein L35Ae [*Carabus gra*... 164	2E-39	J
contig07213	gi|194382944|dbj|BAG59028.1| unnamed protein product [*Homo sapiens*] 166	7E-40	A
contig11370	gi|121512010|gb|ABM55456.1| S10e ribosomal protein [*Xenopsylla c*... 167	6E-40	J
contig00172	gi|114153130|gb|ABI52731.1| 40S ribosomal protein S24 [*Argas mon*... 167	4E-40	J
contig00153	gi|149287152|gb|ABR23475.1| 60s ribosomal protein L34 [*Ornithodo*... 167	3E-40	J
contig05901	gi|170575130|ref|XP_001893112.1| Probable ATP-dependent RNA heli... 168	1E-40	A
contig01043	gi|110757770|ref|XP_393239.3| PREDICTED: similar to Eukaryotic t... 110	8E-41	J
contig01965	gi|60115445|dbj|BAD90013.1| p68 RNA helicase [*Tubifex tubifex*] 169	8E-41	A
contig01870	gi|110766548|ref|XP_623580.2| PREDICTED: similar to eIF3-S8 CG49... 171	5E-41	J
contig00004	gi|159145778|gb|ABW90426.1| putative ribosomal protein S20 [*Bare*... 171	2E-41	J
contig00711	gi|67083893|gb|AAY66881.1| ribosomal protein L27A [*Ixodes scapul*... 171	2E-41	J
contig00451	gi|67083795|gb|AAY66832.1| protein translation factor SUI1-like ... 175	6E-42	J
contig06217	gi|66558122|ref|XP_396907.2| PREDICTED: similar to Nhp2 non-hist... 119	3E-42	AJ
contig12482	gi|149287166|gb|ABR23482.1| 40S ribosomal protein S12 [*Ornithodo*... 177	1E-42	J
contig11987	gi|196476807|gb|ACG76267.1| histone 2B [*Amblyomma americanum*] 178	6E-43	B
contig00706	gi|118083778|ref|XP_001232842.1| PREDICTED: hypothetical protein... 177	3E-43	J
contig02242	gi|112984062|ref|NP_001036824.1| Multiprotein bridging factor 1 ... 179	3E-43	K
contig01190	gi|72159025|ref|XP_790770.1| PREDICTED: similar to ribosomal pro... 179	1E-43	J
contig03232	gi|195051695|ref|XP_001993152.1| GH13236 [*Drosophila grimshawi*] ... 179	1E-43	J
contig09267	gi|115739074|ref|XP_783189.2| PREDICTED: similar to DPH5 homolog... 178	1E-43	J
contig12594	gi|158296283|ref|XP_001237856.2| AGAP006668-PA [*Anopheles gambia*... 183	8E-44	A
contig02863	gi|92081444|dbj|BAE93269.1| RNA helicase [*Tubifex tubifex*] 182	5E-44	A
contig00188	gi|196476807|gb|ACG76267.1| histone 2B [*Amblyomma americanum*] 182	4E-44	B
contig00946	gi|24266986|gb|AAN52389.1| ribosomal protein S17 [*Branchiostoma* ... 180	3E-44	J
contig11433	gi|156550927|ref|XP_001603100.1| PREDICTED: similar to transcrip... 180	3E-44	K
contig03135	gi|54400666|ref|NP_001006082.1| eukaryotic translation initiatio... 183	1E-44	J
contig01347	gi|126321316|ref|XP_001379079.1| PREDICTED: similar to RRS1 ribo... 184	9E-45	J
contig03939	gi|126332314|ref|XP_001376996.1| PREDICTED: similar to nucleolar... 186	4E-46	AJ
contig02046	gi|172051200|gb|ACB70375.1| translation elongation factor EF-1 a... 187	2E-46	J
contig00085	gi|72088524|ref|XP_780039.1| PREDICTED: similar to Ribosomal pro... 188	1E-46	J
contig00529	gi|195453932|ref|XP_002074008.1| GK14409 [*Drosophila willistoni*]... 190	3E-47	K
contig00473	gi|196003590|ref|XP_002111662.1| hypothetical protein TRIADDRAFT... 191	2E-47	AJ
contig02006	gi|195450945|ref|XP_002072700.1| GK13546 [*Drosophila willistoni*]... 194	2E-47	B
contig13040	gi|67083805|gb|AAY66837.1| eukaryotic translation initiation fac... 196	1E-48	J
contig00166	gi|149287034|gb|ABR23416.1| 60S ribosomal protein L44 [*Ornithodo*... 140	6E-49	J
contig00151	gi|51011522|gb|AAT92170.1| ribosomal protein L6 [*Ixodes pacificus*] 199	3E-49	J
contig00003	gi|148704906|gb|EDL36853.1| basic leucine zipper and W2 domains ... 201	2E-49	J
contig11364	gi|149733277|ref|XP_001502820.1| PREDICTED: chromodomain helicas... 197	2E-49	K
contig02310	gi|170053486|ref|XP_001862696.1| Histone H3c [*Culex quinquefasci*... 177	1E-49	B
contig04388	gi|156380038|ref|XP_001631762.1| predicted protein [*Nematostella*... 198	1E-49	A

Contig	BLAST result	e value	Class
contig07684	gi|158286472|ref|XP_308774.4| AGAP006996-PA [*Anopheles gambiae* s... 198	1E-49	J
contig13197	gi|51011530|gb|AAT92174.1| ribosomal protein L30 [*Ixodes pacific*... 198	1E-49	J
contig13246	gi|65332270|gb|AAY42210.1| ribosomal protein L23 [*Haemaphysalis* ... 201	4E-50	J
contig01792	gi|156541180|ref|XP_001600613.1| PREDICTED: similar to histone d... 200	3E-50	B
contig06542	gi|157105268|ref|XP_001648792.1| pre-mrna cleavage factor im, 25... 202	9E-51	A
contig08403	gi|156549876|ref|XP_001603892.1| PREDICTED: similar to ribosomal... 203	4E-51	J
contig03668	gi|157119079|ref|XP_001659326.1| elongation factor tu (ef-tu) [A... 205	3E-51	J
contig07546	gi|91087871|ref|XP_969564.1| PREDICTED: similar to ribosomal pro... 204	3E-51	J
contig00199	gi|114153170|gb|ABI52751.1| 40S ribosomal protein S26 [*Argas mon*... 209	7E-53	J
contig08490	gi|195055839|ref|XP_001994820.1| GH13965 [*Drosophila grimshawi*] ... 211	4E-53	J
contig00384	gi|149286948|gb|ABR23373.1| 40S ribosomal protein S14 [*Ornithodo*... 214	9E-54	J
contig02763	gi|51467966|ref|NP_001003860.1| maternal G10 transcript [*Danio r*... 147	1E-54	K
contig00406	gi|62122748|ref|NP_001014311.1| eukaryotic translation initiatio... 222	1E-55	J
contig12199	gi|91086117|ref|XP_968147.1| PREDICTED: similar to S7e ribosomal... 223	1E-56	J
contig01362	gi|62083461|gb|AAX62455.1| ribosomal protein L13 [*Lysiphlebus te*... 223	8E-57	J
contig12195	gi|149689024|gb|ABR27827.1| 40S ribosomal protein S15/S22 [*Triat*... 227	4E-58	J
contig01756	gi|121952302|sp|Q09JW2.1|RL17_ARGMO 60S ribosomal protein L17 >g... 228	3E-58	J
contig00105	gi|83028352|ref|XP_892120.1| PREDICTED: hypothetical protein [*Mu*... 166	2E-58	J
contig11850	gi|161661035|gb|ABX75384.1| 40s ribosomal protein S15 [*Lycosa si*... 233	6E-60	J
contig02391	gi|149286992|gb|ABR23395.1| ribosomal protein S18 [*Ornithodoros* ... 235	2E-60	J
contig03221	gi|67083925|gb|AAY66897.1| ribosomal protein S16 [*Ixodes scapula*... 235	2E-60	J
contig12746	gi|67083927|gb|AAY66898.1| ribosomal protein L18a [*Ixodes scapul*... 236	1E-60	J
contig03725	gi|48102797|ref|XP_395432.1| PREDICTED: similar to Transcription... 160	3E-61	K
contig11917	gi|197210381|gb|ACH48198.1| translation initiation factor 5A [Or... 241	1E-61	J
contig01003	gi|67084019|gb|AAY66944.1| ribosomal protein L21 [*Ixodes scapula*... 240	4E-62	J
contig00545	gi|67083991|gb|AAY66930.1| ribosomal protein L19 [*Ixodes scapula*... 243	1E-62	J
contig00769	gi|149287142|gb|ABR23470.1| 60s ribosomal protein L18 [*Ornithodo*... 243	1E-62	J
contig13085	gi|156548621|ref|XP_001608164.1| PREDICTED: hypothetical protein... 244	5E-63	B
contig01585	gi|2253411|gb|AAB62936.1| PP2A inhibitor [*Tetraodon fluviatilis*] 251	4E-65	L
contig01300	gi|193647951|ref|XP_001943223.1| PREDICTED: similar to eukaryoti... 251	1E-65	J
contig04011	gi|157118999|ref|XP_001659287.1| DEAD box ATP-dependent RNA heli... 243	5E-66	A
contig00673	gi|149757872|ref|XP_001492034.1| PREDICTED: similar to ribosomal... 255	3E-66	J
contig06564	gi|47228339|emb|CAG07734.1| unnamed protein product [*Tetraodon n*... 254	1E-66	A
contig12581	gi|161661015|gb|ABX75375.1| 60S ribosomal protein L13A [*Lycosa s*... 256	9E-67	J
contig02866	gi|121543837|gb|ABM55583.1| putative 60S ribosomal protein L23 [... 256	6E-67	J
contig12216	gi|156548621|ref|XP_001608164.1| PREDICTED: hypothetical protein... 265	3E-69	B
contig11515	gi|194210878|ref|XP_001491419.2| PREDICTED: similar to histone H... 268	3E-70	B
contig12264	gi|110748814|ref|XP_001120211.1| PREDICTED: similar to arrest CG... 273	2E-71	A
contig00159	gi|61369380|gb|AAX43326.1| ribosomal protein S13 [synthetic cons... 276	5E-73	J
contig00535	gi|67083817|gb|AAY66843.1| ribosomal protein S8 [*Ixodes scapularis*] 278	3E-73	J
contig13097	gi|91089607|ref|XP_967635.1| PREDICTED: similar to S9e ribosomal... 280	9E-74	J
contig00187	gi|41054585|ref|NP_955885.1| eukaryotic translation initiation f... 237	3E-74	J
contig00014	gi|149287182|gb|ABR23490.1| 60S ribosomal protein L9 [*Ornithodor*... 281	2E-74	J
contig01669	gi|66511248|ref|XP_623444.1| PREDICTED: similar to CG5684-PA, is... 223	3E-76	A
contig00229	gi|54039492|sp|Q86FP7|RS23_DERVA 40S ribosomal protein S23 >gnl|... 290	4E-77	J
contig12388	gi|51011506|gb|AAT92162.1| Shwachman-Bodian-Diamond syndrome-lik... 236	2E-82	J
contig11602	gi|67084007|gb|AAY66938.1| ribosomal protein L11 [*Ixodes scapula*... 313	4E-84	J
contig12503	gi|149287168|gb|ABR23483.1| 40S ribosomal protein SA [*Ornithodor*... 316	3E-84	J
contig11880	gi|67083987|gb|AAY66928.1| ribosomal protein L7-like [*Ixodes sca*... 317	7E-85	J
contig00163	gi|12848978|dbj|BAB28159.1| unnamed protein product [*Mus musculus*] 230	2E-85	J
contig02053	gi|115497728|ref|NP_001069059.1| aconitase 1, soluble [*Bos tauru*... 322	3E-86	AJ

Contig	BLAST result	e value	Class
contig13196	gi|47214750|emb|CAG01285.1| unnamed protein product [*Tetraodon n*... 330	1E-88	J
contig13150	gi|67083973|gb|AAY66921.1| ribosomal protein L15 [*Ixodes scapula*... 336	1E-90	J
contig04097	gi|91087957|ref|XP_972849.1| PREDICTED: similar to eIF3-S9 [*Trib*... 337	6E-91	J
contig00008	gi|66533098|ref|XP_397307.2| PREDICTED: similar to Ribosomal pro... 338	2E-91	J
contig01013	gi|67083831|gb|AAY66850.1| 60S acidic ribosomal protein P0 [*Ixod*... 236	2E-91	J
contig00649	gi|91080647|ref|XP_974536.1| PREDICTED: similar to DEAD box ATP-... 345	4E-93	A
contig02376	gi|115735325|ref|XP_798852.2| PREDICTED: similar to Staphylococc... 346	3E-93	K
contig01129	gi|118403668|ref|NP_001072159.1| hypothetical protein LOC447981 ... 348	5E-94	J
contig11833	gi|189238197|ref|XP_001807880.1| PREDICTED: similar to translati... 353	2E-95	J
contig00577	gi|156406016|ref|XP_001641027.1| predicted protein [*Nematostella*... 261	1E-98	AJ
contig01777	gi|195055139|ref|XP_001994478.1| GH17270 [*Drosophila grimshawi*] ... 363	9E-99	A
contig00211	gi|29825585|gb|AAO92286.1| 40S ribosomal protein S5 [*Dermacentor*... 369	1E-100	J
contig00715	gi|67083829|gb|AAY66849.1| 60s ribosomal protein L10 [*Ixodes sca*... 370	1E-101	J
contig11420	gi|91087657|ref|XP_973536.1| PREDICTED: similar to eIF2 alpha su... 373	1E-101	J
contig12622	gi|114153002|gb|ABI52667.1| 40S ribosomal protein S3a [*Argas mon*... 385	1E-105	J
contig12743	gi|156536919|ref|XP_001608023.1| PREDICTED: similar to ribosomal... 300	1E-106	A
contig00763	gi|170059336|ref|XP_001865319.1| eukaryotic translation initiati... 399	1E-109	J
contig00209	gi|149286910|gb|ABR23354.1| 40S ribosomal protein S2/30S [*Ornith*... 407	1E-112	J
contig11568	gi|149689084|gb|ABR27873.1| 60S ribosomal protein L8 [*Triatoma i*... 292	1E-112	J
contig00468	gi|132983|sp|P15125|RL5A_XENLA 60S ribosomal protein L5-A >gnl|B... 411	1E-113	J
contig01885	gi|156392861|ref|XP_001636266.1| predicted protein [*Nematostella*... 419	1E-115	J
contig00327	gi|168983901|emb|CAQ09737.1| HLA-B associated transcript 1 [*Homo*... 426	1E-117	A
contig00250	gi|75029893|sp|Q4PMB3|RS4_IXOSC 40S ribosomal protein S4 >gnl|BL... 431	1E-119	J
contig11256	gi|51965682|emb|CAG29663.1| translation initiation factor 2 gamm... 495	1E-151	J
contig11504	gi|157118999|ref|XP_001659287.1| DEAD box ATP-dependent RNA heli... 484	1E-152	A
contig00656	gi|145974737|gb|ABQ00072.1| PL10A [*Fenneropenaeus chinensis*] 601	1E-169	A
contig01288	gi|66551115|ref|XP_623285.1| PREDICTED: similar to Eukaryotic in... 612	1E-173	J
contig11541	gi|156554128|ref|XP_001604076.1| PREDICTED: similar to poly A bi... 545	1E-177	AJ
contig11801	gi|66566113|ref|XP_624821.1| PREDICTED: similar to Ribosomal pro... 633	1E-179	J
contig00411	gi|66508439|ref|XP_392691.2| PREDICTED: similar to Elongation fa... 1369	0	J
contig10489	gi|125536477|gb|EAY82965.1| hypothetical protein OsI_036924 [*Ory*... 23	0	K
contig12874	gi|40786900|gb|AAR89978.1| putative elongation factor 1-alpha [*H*... 492	0	J
contig00023	No hits found		L
contig00675	No hits found		K
contig02487	No hits found		J
contig02803	No hits found		J
contig02913	No hits found		B
contig03286	No hits found		B
contig03568	No hits found		K
contig04390	No hits found		J
contig04846	No hits found		K
contig05208	No hits found		K
contig05879	No hits found		J
contig06237	No hits found		K
contig06425	No hits found		K
contig06427	No hits found		B
contig06814	No hits found		J
contig06982	No hits found		L
contig07053	No hits found		J
contig07096	No hits found		J
contig07733	No hits found		KL

Contig	BLAST result	e value	Class
contig08265	No hits found		K
contig08384	No hits found		J
contig08412	No hits found		A
contig08927	No hits found		K
contig10054	No hits found		BK
contig10332	No hits found		K
contig10586	No hits found		L
contig11922	No hits found		J
contig12219	No hits found		K
contig12456	No hits found		A
contig12551	No hits found		J
contig12642	No hits found		L
contig12692	No hits found		A
contig13363	No hits found		AR

Information storage and processing and Cellular processes and signaling

Contig	BLAST result	e value	Classification
contig01496	gi|91078036|ref|XP_966313.1| PREDICTED: similar to casein kinase... 388	1E-106	TDK
contig11184	gi|47575810|ref|NP_001001249.1| nucleosome assembly protein 1-li... 225	8E-57	BD
contig04557	gi|91080113|ref|XP_967415.1| PREDICTED: similar to eukaryotic tr... 218	1E-55	JT
contig02965	gi|170649627|gb|ACB21214.1| host cell factor 1 (predicted) [Call... 218	2E-55	DK
contig07437	gi|157134878|ref|XP_001656487.1| protein arginine n-methyltransf... 124	8E-51	OKT
contig07785	gi|193591939|ref|XP_001945590.1| PREDICTED: similar to histone-a... 182	7E-45	OK
contig03451	gi|194377638|dbj|BAG57767.1| unnamed protein product [*Homo sapiens*] 135	8E-31	OK
contig08662	gi|126327443|ref|XP_001367742.1| PREDICTED: similar to replicati... 128	2E-28	DL
contig05679	gi|157110270|ref|XP_001651031.1| mothers against dpp protein [*Ae*... 128	2E-28	TK
contig11538	gi|67084061|gb|AAY66965.1| 40S ribosomal protein S30 [*Ixodes sca*... 112	6E-27	JO
contig09762	gi|47220687|emb|CAG11756.1| unnamed protein product [*Tetraodon n*... 119	8E-26	KT
contig02077	gi|195571923|ref|XP_002103950.1| GD20702 [*Drosophila simulans*] >... 118	1E-25	OKT
contig09011	gi|197215681|gb|ACH53070.1| host cell factor 1 (predicted) [*Otol*... 107	3E-22	DK
contig02837	gi|156379827|ref|XP_001631657.1| predicted protein [*Nematostella*... 101	6E-20	TK
contig03397	gi|27882339|gb|AAH44511.1| CDC5 cell division cycle 5-like (S. p... 97	4E-19	AD
contig08303	gi|170041711|ref|XP_001848597.1| mitosis protein dim1 [*Culex qui*... 88	2E-16	AD
contig07657	gi|114051800|ref|NP_001040433.1| eukaryotic translation initiati... 66	2E-15	JT
contig06403	gi|110758541|ref|XP_001120794.1| PREDICTED: similar to HECT, UBA... 85	2E-15	OK
contig08113	gi|119351872|gb|ABL63639.1| STAT [*Hyphantria cunea*] 81	3E-14	KT
contig02338	gi|48141041|ref|XP_397181.1| PREDICTED: similar to signal transd... 79	1E-13	KT
contig06544	gi|72016481|ref|XP_779912.1| PREDICTED: similar to replication f... 78	2E-13	DL
contig00699	gi|149411128|ref|XP_001515279.1| PREDICTED: similar to Rb1-induc... 75	1E-12	KRT
contig01550	gi|110757972|ref|XP_001120878.1| PREDICTED: similar to delangin ... 74	3E-12	BDL
contig09339	gi|131328778|gb|AAH35143.2| Tigger transposable element derived ... 62	7E-12	BD
contig03005	gi|19920938|ref|NP_609217.1| CG17293 CG17293-PA [*Drosophila mela*... 70	5E-11	ABO
contig09494	gi|1709741|sp|Q11208|PARP_SARPE Poly [ADP-ribose] polymerase (PA... 69	1E-10	KLO
contig06844	gi|110749415|ref|XP_624477.2| PREDICTED: similar to Poly [ADP-ri... 67	3E-10	KLO
contig07159	gi|154273336|ref|XP_001537520.1| hypothetical protein HCAG_07829... 65	1E-09	BD
contig01721	gi|51889790|gb|AAU12852.1| GCIP-interacting protein [*Xenopus tro*... 63	6E-09	DA
contig06563	gi|115625818|ref|XP_785475.2| PREDICTED: similar to CK2 alpha su... 62	2E-08	TDK
contig07788	gi|115496982|ref|NP_001069134.1| microspherule protein 1 [*Bos ta*... 60	6E-08	KT
contig10266	gi|48141041|ref|XP_397181.1| PREDICTED: similar to signal transd... 59	1E-07	KT
contig00718	gi|194669355|ref|XP_585794.4| PREDICTED: structural maintenance ... 57	6E-07	BDL
contig02422	gi|154937332|ref|NP_001073186.1| ubinuclein 1 [*Gallus gallus*] 57	6E-07	KT
contig05571	gi|170053161|ref|XP_001862547.1| poly [*Culex quinquefasciatus*] >... 51	3E-05	KLO
contig09156	gi|115757018|ref|XP_001199118.1| PREDICTED: hypothetical protein... 50	4E-05	KLO
contig01740	gi|195135210|ref|XP_002012027.1| GI16655 [*Drosophila mojavensis*]... 47	5E-04	AD
contig03503	gi|91081917|ref|XP_970376.1| PREDICTED: similar to chromaffin gr... 45	0.002	BD
contig10161	gi|170590714|ref|XP_001900116.1| SMC proteins Flexible Hinge Dom... 45	0.002	BD
contig05296	gi|189241940|ref|XP_971495.2| PREDICTED: similar to Serine-prote... 42	0.016	TBLD
contig02557	gi|156546691|ref|XP_001604244.1| PREDICTED: similar to ENSANGP00... 41	0.026	OJ
contig09786	gi|194745504|ref|XP_001955228.1| GF16341 [*Drosophila ananassae*] ... 41	0.035	KRT
contig13221	gi|85857568|gb|ABC86319.1| IP15837p [*Drosophila melanogaster*] 44	0.037	BD
contig09502	gi|170064093|ref|XP_001867382.1| myosin I [*Culex quinquefasciatu*... 40	0.059	AD
contig06627	gi|111026197|ref|YP_708480.1| acyl-CoA transferase, N-terminal [... 41	0.073	KTDL
contig10956	gi|50545761|ref|XP_500419.1| hypothetical protein [*Yarrowia lipo*... 40	0.078	DK
contig12823	gi|170574743|ref|XP_001892943.1| Heat shock factor binding prote... 39	0.130	KO
contig07810	gi|159037567|ref|YP_001536820.1| hypothetical protein Sare_1944 ... 37	0.38	TK
contig02489	gi|51556463|ref|YP_068096.1| rh02 [*Cercopithecine herpesvirus* 8]... 37	0.5	TK

Contig	BLAST result	e value	Classification
contig01636	gi|18420415|ref|NP_568409.1| La domain-containing protein / prol... 37	0.650	OJ
contig12931	gi|194678652|ref|XP_596969.4| PREDICTED: similar to Nuclear enve... 36	0.860	BD
contig13091	gi|194678652|ref|XP_596969.4| PREDICTED: similar to Nuclear enve... 36	1.100	BD
contig00281	gi|3641289|gb|AAC36334.1| RF-C/activator 1 homolog [*Homo sapiens*] 36	1.100	DL
contig02982	gi|119586334|gb|EAW65930.1| neuronal PAS domain protein 3 [Homo ... 35	1.9	TK
contig01827	gi|158296679|ref|XP_317030.3| AGAP008417-PA [*Anopheles gambiae* s... 34	4.300	DKL

Information storage and processing, Cellular processes and signaling and Metabolism

Contig	BLAST result		e value	Class
contig10844	gi|149732126|ref|XP_001497982.1| PREDICTED: similar to Inositol ...	35	2.500	KIT
contig04618	gi|170066943|ref|XP_001868285.1| amidase [*Culex quinquefasciatus*...	44	0.004	JIT
contig05502	gi|156369693|ref|XP_001628109.1| predicted protein [*Nematostella*...	56	8E-07	JIT
contig02010	No hits found			OKIT
contig05236	No hits found			OKIT
contig05479	No hits found			KIT

Information storage and processing and Metabolism

Contig	BLAST result		e value	Class
contig07805	gi|162287117|ref|NP_001104751.1| wu:fb18g03 [*Danio rerio*] >gnl|B...	35	2.4	EJ
contig09433	gi|118095225|ref|XP_422757.2| PREDICTED: similar to ATP-binding ...	99	1E-19	EJ

Metabolism

Contig	BLAST result	e value	Class
contig06672	gi|170761567|ref|YP_001787246.1| integrase [*Clostridium botulinu*... 33	9.600	C
contig04226	gi|167627306|ref|YP_001677806.1| glycosyltransferase [*Francisell*... 33	9.400	C
contig06618	gi|5882239|gb|AAD55259.1|AF159117_1 thermal hysteresis protein i... 33	9.400	C
contig08779	gi|6323274|ref|NP_013346.1| Cytidine deaminase; catalyzes the mo... 33	9.400	F
contig05089	gi|195029375|ref|XP_001987549.1| GH19904 [*Drosophila grimshawi*] ... 33	9.300	E
contig08674	gi|195337485|ref|XP_002035359.1| GM14667 [*Drosophila sechellia*] ... 33	9.200	C
contig00432	gi|15239403|ref|NP_197919.1| PLDALPHA3 (PHOSPHLIPASE D ALPHA 3);... 33	7.300	I
contig13202	gi|154335645|ref|XP_001564061.1| hypothetical protein LbrM18_V2.... 33	7.300	C
contig00831	gi|163915075|ref|NP_001106519.1| hypothetical protein LOC1001277... 33	7.200	C
contig01235	gi|41059092|gb|AAR99079.1| xanthine dehydrogenase 2 [*Arabidopsis*... 33	7.200	IG
contig01523	gi|195439294|ref|XP_002067566.1| GK16499 [*Drosophila willistoni*]... 33	7.200	C
contig03390	gi|194043532|ref|XP_001926169.1| PREDICTED: chloride channel 3 [... 33	7.200	I
contig03856	gi|194913607|ref|XP_001982736.1| GG16385 [*Drosophila erecta*] >gn... 33	7.200	P
contig08408	gi|47226520|emb|CAG08536.1| unnamed protein product [*Tetraodon n*... 33	7.200	I
contig09523	gi|119566859|gb|ABL84267.1| TadZ [*Yersinia ruckeri*] 33	7.200	E
contig09655	gi|149917886|ref|ZP_01906381.1| Serine/Threonine protein kinase ... 33	7.200	H
contig10882	gi|88810943|ref|ZP_01126199.1| ribulose-bisphosphate carboxylase... 33	7.200	Q
contig04411	gi|167388184|ref|XP_001738464.1| hypothetical protein EDI_097870... 33	7.100	E
contig11876	gi|110189777|gb|ABG56071.1| hexokinase 2 [*Monocercomonoides* sp. ... 33	7.100	I
contig13011	gi|148557627|ref|YP_001265209.1| adenosylcobyric acid synthase (... 33	7.100	G
contig11863	gi|47211517|emb|CAF90313.1| unnamed protein product [*Tetraodon n*... 27	6.800	I
contig07332	gi|169212696|ref|XP_001716936.1| PREDICTED: hypothetical protein... 33	5.600	Q
contig01793	gi|110772525|ref|XP_625192.2| PREDICTED: similar to CG5205-PA, p... 33	5.500	P
contig02632	gi|117959924|ref|NP_963912.1| GNAS complex locus isoform e [*Mus* ... 33	5.500	G
contig03350	gi|194037217|ref|XP_001924600.1| PREDICTED: similar to phospholi... 33	5.500	C
contig03911	gi|78211722|ref|YP_380501.1| putative glycosyl transferase, grou... 33	5.500	G
contig06287	gi|118382431|ref|XP_001024373.1| hypothetical protein TTHERM_005... 33	5.500	I
contig09701	gi|171322066|ref|ZP_02910938.1| Mammalian cell entry related dom... 33	5.500	C
contig09758	gi|124001539|ref|NP_001074219.1| SRY-box containing gene 7 [*Dani*... 33	5.500	G
contig01909	gi|56551058|ref|YP_161897.1| TolR biopolymer transport protein [... 33	5.400	I
contig08109	gi|170024821|ref|YP_001721326.1| hypothetical protein YPK_2596 [... 33	5.400	F
contig01663	gi|73953846|ref|XP_868075.1| PREDICTED: similar to delangin isof... 34	4.300	P
contig06178	gi|195112532|ref|XP_002000826.1| GI10446 [*Drosophila mojavensis*]... 34	4.300	I
contig03038	gi|149027168|gb|EDL82892.1| rCG41735, isoform CRA_a [*Rattus norv*... 34	4.200	I
contig05640	gi|149493002|ref|XP_001513425.1| PREDICTED: similar to PANK2 pro... 34	4.200	I
contig07407	gi|86608148|ref|YP_476910.1| cytochrome oxidase assembly protein... 34	4.200	Q
contig08875	gi|89094170|ref|ZP_01167113.1| organic solvent tolerance protein... 34	4.200	Q
contig04719	gi|170106387|ref|XP_001884405.1| predicted protein [*Laccaria bic*... 35	3.500	C
contig05367	gi|162147649|ref|YP_001602110.1| putative ribosomal RNA large su... 34	3.300	E
contig09282	gi|15230521|ref|NP_190069.1| lecithin:cholesterol acyltransferas... 34	3.200	G
contig10179	gi|193216946|ref|YP_002000188.1| massive surface protein MspK [*M*... 34	3.200	C
contig12713	gi|187956956|gb|AAI57934.1| D6Ertd527e protein [*Mus musculus*] 34	3.200	F
contig12861	gi|158316180|ref|YP_001508688.1| hypothetical protein Franean1_4... 35	3.100	C
contig00843	gi|194875784|ref|XP_001973664.1| GG16212 [*Drosophila erecta*] >gn... 35	2.500	C
contig02343	gi|193209775|ref|NP_510181.3| F09A5.1 [*Caenorhabditis elegans*] >... 35	2.500	E
contig04093	gi|4895195|gb|AAD32782.1|AC007661_19 putative mitochondrial carr... 35	2.500	P
contig04235	gi|91794002|ref|YP_563653.1| hypothetical protein Sden_2651 [*She*... 35	2.500	C
contig12826	gi|198145521|gb|EDY72139.1| GA22932 [*Drosophila pseudoobscura* ps... 35	2.500	I
contig07870	gi|170586182|ref|XP_001897858.1| Guanylate kinase family protein... 35	2.400	F

Contig	BLAST result	e value	Class
contig09854	gi|72393371|ref|XP_847486.1| hypothetical protein, conserved [*Tr*... 35	1.900	I
contig12422	gi|6321844|ref|NP_011920.1| Metallothionein, binds copper and me... 35	1.900	P
contig13067	gi|74004821|ref|XP_545512.2| PREDICTED: similar to ATP-binding c... 36	1.900	Q
contig02238	gi|168039262|ref|XP_001772117.1| predicted protein [*Physcomitrel*... 35	1.500	F
contig06690	gi|189523697|ref|XP_001341635.2| PREDICTED: im:6911926 [*Danio re*... 35	1.500	P
contig07035	gi|50290817|ref|XP_447841.1| unnamed protein product [*Candida gl*... 35	1.500	G
contig08089	gi|111020072|ref|YP_703044.1| MerR family transcriptional regula... 35	1.500	P
contig10365	gi|156367570|ref|XP_001627489.1| predicted protein [*Nematostella*... 35	1.500	I
contig12208	gi|158296537|ref|XP_316929.4| AGAP008512-PA [*Anopheles gambiae* s... 35	1.500	IR
contig04848	gi|170109773|ref|XP_001886093.1| predicted protein [*Laccaria bic*... 35	1.400	E
contig10181	gi|54024975|ref|YP_119217.1| acetolactate synthase 1 catalytic s... 35	1.400	E
contig11536	gi|160941702|ref|ZP_02089029.1| hypothetical protein CLOBOL_0659... 35	1.400	C
contig00603	gi|77553801|gb|ABA96597.1| MYB10, putative, expressed [*Oryza sat*... 36	1.100	G
contig02434	gi|67043765|gb|AAY63981.1| 3-hydroxyacyl-CoA dehydrogenase [*Lysi*... 36	1.100	G
contig04671	gi|125583648|gb|EAZ24579.1| hypothetical protein OsJ_008062 [*Ory*... 36	1.100	C
contig07763	gi|194217761|ref|XP_001488176.2| PREDICTED: similar to serine hy... 36	1.100	C
contig07962	gi|189238031|ref|XP_966733.2| PREDICTED: similar to T21C9.6 [*Tri*... 36	1.100	E
contig09972	gi|47220907|emb|CAG03114.1| unnamed protein product [*Tetraodon n*... 36	1.100	P
contig04394	gi|189240635|ref|XP_969354.2| PREDICTED: similar to AGAP009835-P... 36	0.860	I
contig04588	gi|116788431|gb|ABK24877.1| unknown [*Picea sitchensis*] 36	0.860	E
contig09050	gi|57109426|ref|XP_549598.1| PREDICTED: hypothetical protein XP_... 36	0.860	C
contig11461	gi|152965329|ref|YP_001361113.1| EPSP synthase (3-phosphoshikima... 36	0.850	C
contig00359	gi|189195286|ref|XP_001933981.1| DNA polymerase alpha accessory ... 36	0.840	I
contig04928	gi|134293884|ref|YP_001117620.1| glutamate dehydrogenase (NAD) [... 36	0.840	P
contig04935	gi|194564602|ref|YP_002099624.1| Coenzyme F420-dependent N5,N10-... 36	0.840	F
contig04495	gi|157134805|ref|XP_001656451.1| isocitrate dehydrogenase [Aedes... 37	0.650	E
contig07260	gi|195037280|ref|XP_001990092.1| GH18426 [*Drosophila grimshawi*] ... 37	0.650	C
contig08539	gi|193690532|ref|XP_001942614.1| PREDICTED: similar to ribosomal... 37	0.650	G
contig02996	gi|195376021|ref|XP_002046795.1| GJ12289 [*Drosophila virilis*] >g... 39	0.530	Q
contig00194	gi|169596476|ref|XP_001791662.1| hypothetical protein SNOG_01001... 37	0.520	GE
contig09012	gi|45361643|ref|NP_989397.1| ER degradation enhancer, mannosidas... 37	0.510	P
contig03048	gi|45384172|ref|NP_990417.1| ferritin, heavy polypeptide 1 [*Gall*... 37	0.500	E
contig00135	gi|186972934|pdb|2Z9A|A Chain A, Crystal Structure Of Human Sapo... 37	0.390	I
contig06391	gi|195118426|ref|XP_002003738.1| GI18075 [*Drosophila mojavensis*]... 37	0.390	C
contig11274	gi|157356366|emb|CAO62518.1| unnamed protein product [*Vitis vini*... 37	0.390	C
contig02954	gi|195396455|ref|XP_002056847.1| GJ16668 [*Drosophila virilis*] >g... 37	0.380	G
contig03828	gi|86747726|ref|YP_484222.1| translation initiation factor IF-2 ... 37	0.380	C
contig07010	gi|195384738|ref|XP_002051069.1| GJ14144 [*Drosophila virilis*] >g... 37	0.380	FE
contig08736	gi|149634602|ref|XP_001513273.1| PREDICTED: similar to SNARE Vti... 37	0.380	EG
contig09127	gi|195486210|ref|XP_002091408.1| GE12257 [*Drosophila yakuba*] >gn... 37	0.380	C
contig11873	gi|196015978|ref|XP_002117844.1| hypothetical protein TRIADDRAFT... 39	0.290	F
contig00177	gi|195457363|ref|XP_002075541.1| GK14532 [*Drosophila willistoni*]... 38	0.230	I
contig02068	gi|193808552|emb|CAQ39255.1| hypothetical protein, conserved in ... 38	0.220	P
contig02504	gi|148745279|gb|AAI42446.1| GLTP protein [*Bos taurus*] 38	0.220	I
contig01462	gi|156355189|ref|XP_001623555.1| predicted protein [*Nematostella*... 39	0.170	G
contig02587	gi|114663857|ref|XP_523518.2| PREDICTED: malonyl-CoA decarboxyla... 39	0.170	G
contig03020	gi|118777586|ref|XP_308162.3| AGAP007714-PA [*Anopheles gambiae* s... 39	0.130	C
contig03369	gi|66535318|ref|XP_623221.1| PREDICTED: similar to CG31522-PA, i... 39	0.130	F
contig03536	gi|66766200|dbj|BAD99106.1| divalent metal transporter [*Mizuhope*... 30	0.130	P
contig04357	gi|11499248|ref|NP_070486.1| hypothetical protein AF1658 [*Archae*... 39	0.130	C
contig05886	gi|86147601|ref|ZP_01065911.1| hypothetical protein MED222_22968... 39	0.130	CR

Contig	BLAST result	e value	Class
contig06052	gi|88706159|ref|ZP_01103866.1| Acyl-CoA dehydrogenase, short-cha... 39	0.130	P
contig03758	gi|194373881|dbj|BAG62253.1| unnamed protein product [*Homo sapiens*] 39	0.100	F
contig04602	gi|193713559|ref|XP_001945410.1| PREDICTED: similar to transmemb... 39	0.100	G
contig10314	gi|195486388|ref|XP_002091488.1| GE12215 [*Drosophila yakuba*] >gn... 39	0.100	I
contig11270	gi|1914843|gb|AAC09470.1| lysosomal alpha-mannosidase [*Mus muscu*... 39	0.100	H
contig01689	gi|194766720|ref|XP_001965472.1| GF22508 [*Drosophila ananassae*] ... 39	0.099	F
contig11710	gi|169206246|ref|XP_001716022.1| PREDICTED: hypothetical protein... 39	0.099	IR
contig03433	gi|187597787|ref|ZP_02984044.1| hypothetical protein GdiaDRAFT_2... 40	0.091	I
contig04899	gi|194335007|ref|YP_002016867.1| cyclase family protein [*Prosthe*... 40	0.078	I
contig07423	gi|193659829|ref|XP_001942654.1| PREDICTED: similar to AGAP01194... 40	0.078	C
contig07649	gi|53791863|dbj|BAD53949.1| unknown protein [*Oryza sativa Japoni*... 40	0.078	C
contig02952	gi|1345958|sp|P12276|FAS_CHICK Fatty acid synthase [Includes: [A... 40	0.077	E
contig09134	gi|149643093|ref|NP_001092606.1| hypothetical protein LOC614494 ... 40	0.077	C
contig05145	gi|186685256|ref|YP_001868452.1| phosphorylase kinase alphabeta ... 40	0.076	QI
contig00012	gi|45826276|gb|AAS77782.1| NADH dehydrogenase subunit 3 [*Tetrale*... 40	0.059	F
contig01454	gi|190338968|gb|AAI63342.1| Ela2l protein [*Danio rerio*] 40	0.059	G
contig12058	gi|156361167|ref|XP_001625391.1| predicted protein [*Nematostella*... 40	0.051	E
contig01476	gi|170097792|ref|XP_001880115.1| UTP-glucose-1-phosphate uridyly... 40	0.045	E
contig02794	gi|149240315|ref|XP_001526033.1| hypothetical protein LELG_02591... 42	0.045	F
contig00313	gi|50812392|gb|AAT81406.1| fatty acid elongase [*Gadus morhua*] 42	0.043	P
contig00244	gi|189237128|ref|XP_972994.2| PREDICTED: similar to sodium bicar... 41	0.034	E
contig01980	gi|158292089|ref|XP_313650.4| AGAP004367-PA [*Anopheles gambiae* s... 41	0.034	P
contig08378	gi|149021392|gb|EDL78855.1| rCG59057, isoform CRA_b [*Rattus norv*... 41	0.034	C
contig08810	gi|195115445|ref|XP_002002267.1| GI17291 [*Drosophila mojavensis*]... 41	0.027	IG
contig05627	gi|195126769|ref|XP_002007843.1| GI12152 [*Drosophila mojavensis*]... 41	0.026	G
contig12663	gi|193610823|ref|XP_001951459.1| PREDICTED: similar to CG10320 C... 42	0.024	G
contig03292	gi|17558842|ref|NP_503846.1| CYtochrome P450 family member (cyp-... 33	0.020	G
contig03591	gi|11878241|gb|AAG40862.1| ribonucleotide reductase [*Emericella* ... 42	0.020	E
contig01785	gi|68509955|gb|AAY98350.1| acetoacyl-CoA synthetase [*Amanita mus*... 42	0.016	P
contig00684	gi|192910870|gb|ACF06543.1| uridylate kinase [*Elaeis guineensis*] 42	0.015	G
contig00900	gi|66521130|ref|XP_396194.2| PREDICTED: similar to ATPase type 1... 42	0.015	E
contig02802	gi|196009740|ref|XP_002114735.1| hypothetical protein TRIADDRAFT... 42	0.015	I
contig02852	gi|163914443|ref|NP_001106306.1| hypothetical protein LOC1001272... 42	0.015	E
contig03204	gi|91091362|ref|XP_972603.1| PREDICTED: similar to lethal 35Di [... 43	0.009	Q
contig03487	gi|195474149|ref|XP_002089354.1| GE19067 [*Drosophila yakuba*] >gn... 43	0.009	G
contig04215	gi|119911728|ref|XP_869690.2| PREDICTED: similar to solute carri... 43	0.009	C
contig08595	gi|191637919|ref|YP_001987085.1| Alpha-glucosidase [*Lactobacillu*... 43	0.009	C
contig00967	gi|115614636|ref|XP_001199974.1| PREDICTED: similar to solute ca... 43	0.007	H
contig05142	gi|158292101|ref|XP_313656.4| AGAP004373-PA [*Anopheles gambiae* s... 43	0.007	I
contig07690	gi|147900452|ref|NP_001082222.1| pancreatic elastase 1 [*Xenopus* ... 43	0.007	P
contig00412	gi|148673609|gb|EDL05556.1| mCG4140 [*Mus musculus*] 44	0.005	F
contig03328	gi|156368173|ref|XP_001627570.1| predicted protein [*Nematostella*... 40	0.005	C
contig04756	gi|195587367|ref|XP_002083436.1| GD13354 [*Drosophila simulans*] >... 44	0.005	F
contig05875	gi|157114275|ref|XP_001658020.1| malic enzyme [*Aedes aegypti*] >g... 44	0.004	G
contig10791	gi|55736035|gb|AAV63544.1| fed tick salivary protein 10 [*Ixodes* ... 44	0.004	E
contig07170	gi|47227771|emb|CAG08934.1| unnamed protein product [*Tetraodon n*... 44	0.003	P
contig08624	gi|115615300|ref|XP_791028.2| PREDICTED: similar to FLJ10769 pro... 40	0.003	C
contig08650	gi|166157941|ref|NP_001107380.1| hypothetical protein LOC1001352... 44	0.003	C
contig03078	gi|156230054|gb|AAI52204.1| Elovl4 protein [*Danio rerio*] >gnl|BL... 45	0.002	C
contig06729	gi|52355639|gb|AAU44969.1| retinal short-chain dehydrogenase/red... 45	0.002	E
contig08462	gi|58387888|ref|XP_315885.2| AGAP005860-PA [*Anopheles gambiae* st... 45	0.002	EF

Contig	BLAST result	e value	Class
contig08951	gi|126337907|ref|XP_001365492.1| PREDICTED: similar to cytosolic... 45	0.002	E
contig11309	gi|196007390|ref|XP_002113561.1| hypothetical protein TRIADDRAFT... 45	0.002	G
contig11915	gi|68086161|gb|AAH98178.1| MGC132021 protein [*Xenopus laevis*] 45	0.002	C
contig01732	gi|17506935|ref|NP_492149.1| Temporarily Assigned Gene name fami... 46	0.001	I
contig03199	gi|115960648|ref|XP_001178059.1| PREDICTED: similar to Ubiquinol... 46	0.001	C
contig04906	gi|114051197|ref|NP_001039492.1| thromboxane A synthase 1 (plate... 45	0.001	I
contig06161	gi|148222486|ref|NP_001087043.1| cytochrome P450, family 2, subf... 45	0.001	H
contig07067	gi|58384648|ref|XP_313383.2| AGAP003623-PA [*Anopheles gambiae* st... 45	0.001	CE
contig08525	gi|119473973|ref|XP_001258862.1| short-chain dehydrogenase [*Neos*... 45	0.001	G
contig11638	gi|157352135|emb|CAO43142.1| unnamed protein product [*Vitis vini*... 46	0.001	E
contig13029	gi|188572677|ref|YP_001911141.1| hypothetical protein CBPV_s2gp3... 48	0.001	P
contig13256	gi|196187308|gb|EDX82284.1| AMP-binding enzyme, putative [*Brevun*... 46	0.001	E
contig00780	gi|157123334|ref|XP_001660121.1| oviductin [*Aedes aegypti*] >gnl|... 46	8E-04	P
contig00965	gi|71066248|ref|YP_264975.1| glutathione reductase [*Psychrobacte*... 46	8E-04	G
contig01131	gi|66517199|ref|XP_624588.1| PREDICTED: similar to CG31860-PA [A... 46	8E-04	P
contig07340	gi|170062345|ref|XP_001866628.1| elongase [*Culex quinquefasciatu*... 46	8E-04	I
contig11645	gi|156351329|ref|XP_001622462.1| predicted protein [*Nematostella*... 46	8E-04	E
contig12824	gi|195995563|ref|XP_002107650.1| expressed hypothetical protein ... 47	7E-04	P
contig08337	gi|386960|gb|AAA36329.1| GT mitochondrial solute carrier protein... 40	6E-04	G
contig08970	gi|110757192|ref|XP_625219.2| PREDICTED: similar to deoxyribonuc... 47	6E-04	I
contig11704	gi|51557673|gb|AAU06476.1| cytochrome c oxidase subunit III [Cul... 47	6E-04	GC
contig01302	gi|148222892|ref|NP_001082410.1| similar to inosine 5'-phosphate... 47	5E-04	C
contig07001	gi|119112640|ref|XP_317733.3| AGAP007780-PA [*Anopheles gambiae* s... 47	5E-04	I
contig09509	gi|66565375|ref|XP_395198.2| PREDICTED: similar to CG1597-PA iso... 47	5E-04	G
contig10025	gi|66772913|gb|AAY55767.1| IP10521p [*Drosophila melanogaster*] 47	5E-04	E
contig10546	gi|156546442|ref|XP_001607215.1| PREDICTED: similar to ENSANGP00... 47	5E-04	E
contig03017	gi|196014088|ref|XP_002116904.1| hypothetical protein TRIADDRAFT... 47	4E-04	F
contig04185	gi|66555130|ref|XP_394934.2| PREDICTED: similar to SCAP CG33131-... 47	4E-04	E
contig04427	gi|56753241|gb|AAW24830.1| SJCHGC04571 protein [*Schistosoma japo*... 47	4E-04	E
contig04612	gi|197632233|gb|ACH70840.1| ubiquinol-cytochrome c reductase cor... 41	4E-04	G
contig08425	gi|170030261|ref|XP_001843008.1| elongation of very long chain f... 47	4E-04	G
contig08463	gi|159184758|ref|NP_354438.2| branched-chain alpha-keto acid deh... 47	4E-04	G
contig10521	gi|189524371|ref|XP_001919145.1| PREDICTED: holocarboxylase synt... 48	4E-04	P
contig01388	gi|126311342|ref|XP_001381718.1| PREDICTED: similar to QPS1 [*Mon*... 48	3E-04	C
contig11429	gi|144899161|emb|CAM76025.1| Pyruvate carboxyltransferase [*Magne*... 48	3E-04	IE
contig03513	gi|169849301|ref|XP_001831354.1| hypothetical protein CC1G_00901... 49	1E-04	I
contig04404	gi|196181175|gb|EDX76164.1| O-methyltransferase superfamily [Mic... 49	1E-04	C
contig04863	gi|115753229|ref|XP_001180582.1| PREDICTED: hypothetical protein... 49	1E-04	P
contig06903	gi|145233995|ref|XP_001400370.1| hypothetical protein An02g12420... 49	1E-04	F
contig09375	gi|195969368|gb|ACG60513.1| chitinase 1 precursor [*Litopenaeus v*... 49	1E-04	G
contig02486	gi|145352581|ref|XP_001420619.1| predicted protein [*Ostreococcus*... 50	7E-05	I
contig04881	gi|156369646|ref|XP_001628086.1| predicted protein [*Nematostella*... 50	7E-05	Q
contig05769	gi|156548308|ref|XP_001602653.1| PREDICTED: similar to ctl2 [*Nas*... 50	6E-05	I
contig08213	gi|47498070|ref|NP_998829.1| glutamic-oxaloacetic transaminase 1... 50	6E-05	G
contig08932	gi|195055871|ref|XP_001994836.1| GH17458 [*Drosophila grimshawi*] ... 50	6E-05	F
contig11620	gi|149287114|gb|ABR23456.1| cytochrome c oxidase polyprotein IV ... 50	6E-05	C
contig02994	gi|110762638|ref|XP_624101.2| PREDICTED: similar to small wing C... 50	4E-05	H
contig03632	gi|115683649|ref|XP_788713.2| PREDICTED: similar to MGC81887 pro... 50	4E-05	I
contig05593	gi|156377879|ref|XP_001630873.1| predicted protein [*Nematostella*... 50	4E-05	Q
contig06954	gi|170052086|ref|XP_001862062.1| multidrug resistance-associated... 50	4E-05	E
contig09280	gi|126334883|ref|XP_001375324.1| PREDICTED: hypothetical protein... 50	4E-05	Q

Contig	BLAST result	e value	Class
contig00970	gi|198131481|gb|EAL27496.2| GA19830 [*Drosophila pseudoobscura* ps... 51	3E-05	F
contig01834	gi|194754579|ref|XP_001959572.1| GF11978 [*Drosophila ananassae*] ... 51	3E-05	C
contig03633	gi|116245613|ref|XP_001230565.1| AGAP012661-PA [*Anopheles gambia*... 51	3E-05	G
contig06414	gi|52219050|ref|NP_001004598.1| hypothetical protein LOC447859 [... 51	3E-05	C
contig09808	gi|195022723|ref|XP_001985629.1| GH14400 [*Drosophila grimshawi*] ... 51	3E-05	I
contig00802	gi|189234287|ref|XP_970030.2| PREDICTED: similar to isocitrate d... 52	2E-05	E
contig08182	gi|156139061|ref|NP_001095847.1| adenylosuccinate lyase [*Bos tau*... 40	2E-05	G
contig09976	gi|91080223|ref|XP_972420.1| PREDICTED: similar to 1-acylglycero... 52	2E-05	G
contig10768	gi|170596384|ref|XP_001902745.1| Dihydroorotate dehydrogenase, m... 52	2E-05	G
contig10808	gi|91088621|ref|XP_967480.1| PREDICTED: similar to voltage-depen... 52	2E-05	G
contig03173	gi|50540382|ref|NP_001002657.1| hypothetical protein LOC436930 [... 52	1E-05	F
contig04527	gi|158290080|ref|XP_558852.3| AGAP003398-PA [*Anopheles gambiae* s... 52	1E-05	C
contig04734	gi|90577971|ref|ZP_01233782.1| glycine dehydrogenase [*Vibrio ang*... 47	1E-05	G
contig04791	gi|47227950|emb|CAF97579.1| unnamed protein product [*Tetraodon n*... 52	1E-05	I
contig05114	gi|156393384|ref|XP_001636308.1| predicted protein [*Nematostella*... 52	1E-05	E
contig06277	gi|197098712|ref|NP_001124971.1| phenylalanine hydroxylase [*Pong*... 52	1E-05	C
contig07960	gi|193711493|ref|XP_001951913.1| PREDICTED: similar to CG18317 C... 52	1E-05	C
contig08373	gi|118090097|ref|XP_001232415.1| PREDICTED: hypothetical protein... 52	1E-05	C
contig00635	gi|45386071|gb|AAS59853.1| NPC1 precursor [*Ornithodoros moubata*] 53	9E-06	C
contig04088	gi|194674931|ref|XP_608091.3| PREDICTED: chymotrypsinogen B1 [Bo... 53	9E-06	EG
contig06409	gi|91090780|ref|XP_966783.1| PREDICTED: similar to calcium-trans... 53	9E-06	RP
contig08556	gi|115689712|ref|XP_794215.2| PREDICTED: hypothetical protein, p... 53	7E-06	Q
contig10838	gi|156551161|ref|XP_001605023.1| PREDICTED: similar to ENSANGP00... 53	7E-06	G
contig12696	gi|170745006|ref|YP_001766464.1| hemagluttinin domain-containing... 53	7E-06	C
contig00927	gi|195440466|ref|XP_002068063.1| GK10599 [*Drosophila willistoni*]... 54	5E-06	G
contig02277	gi|195431427|ref|XP_002063743.1| GK15833 [*Drosophila willistoni*]... 54	5E-06	F
contig06917	gi|156543262|ref|XP_001606750.1| PREDICTED: similar to conserved... 54	5E-06	E
contig07160	gi|115659660|ref|XP_001199609.1| PREDICTED: similar to prosaposi... 54	5E-06	H
contig08972	gi|162287393|ref|NP_001104713.1| hypothetical protein LOC1000030... 54	5E-06	F
contig07836	gi|66511507|ref|XP_623995.1| PREDICTED: similar to CG9307-PA iso... 54	4E-06	H
contig08832	gi|114051868|ref|NP_001040436.1| hydroxysteroid dehydrogenase [*B*... 54	4E-06	E
contig10505	gi|94499123|ref|ZP_01305661.1| short-chain alcohol dehydrogenase... 54	4E-06	C
contig11311	gi|56756485|gb|AAW26415.1| SJCHGC09157 protein [*Schistosoma japo*... 54	4E-06	F
contig02808	gi|67083949|gb|AAY66909.1| ATP synthase H+ transporting, mitocho... 55	3E-06	C
contig00430	gi|91081589|ref|XP_975344.1| PREDICTED: similar to AGAP011940-PA... 56	2E-06	Q
contig00855	gi|116004575|ref|NP_001070647.1| hypothetical protein LOC570761 ... 55	2E-06	I
contig01851	gi|193591736|ref|XP_001948381.1| PREDICTED: similar to xaa-pro a... 55	2E-06	G
contig01883	gi|117558471|gb|AAI25766.1| LOC548740 protein [*Xenopus tropicalis*] 55	2E-06	E
contig07758	gi|196012622|ref|XP_002116173.1| hypothetical protein TRIADDRAFT... 55	2E-06	F
contig10267	gi|195161561|ref|XP_002021631.1| GL26614 [*Drosophila persimilis*]... 55	2E-06	I
contig09767	gi|47222031|emb|CAG08286.1| unnamed protein product [*Tetraodon n*... 56	1E-06	G
contig03474	gi|114052669|ref|NP_001040545.1| beta-1,3-galactosyltransferase ... 48	9E-07	I
contig01989	gi|158293995|ref|XP_315341.4| AGAP005325-PA [*Anopheles gambiae* s... 56	8E-07	H
contig02109	gi|115685846|ref|XP_001184955.1| PREDICTED: similar to GA15058-P... 56	8E-07	I
contig05273	gi|114052613|ref|NP_001040544.1| transaldolase [*Bombyx mori*] >gn... 56	8E-07	I
contig06770	gi|47222915|emb|CAF99071.1| unnamed protein product [*Tetraodon n*... 41	7E-07	E
contig03760	gi|58268158|ref|XP_571235.1| diacylglycerol O-acyltransferase [*C*... 57	6E-07	C
contig06156	gi|170043543|ref|XP_001849443.1| 1-phosphatidylinositol-4,5-bisp... 57	6E-07	E
contig06033	gi|13162347|ref|NP_077067.1| ferredoxin reductase [*Rattus norveg*... 57	5E-07	P
contig07513	gi|195571587|ref|XP_002103784.1| GD18795 [*Drosophila simulans*] >... 50	5E-07	I
contig05486	gi|194765045|ref|XP_001964638.1| GF23289 [*Drosophila ananassae*] ... 57	4E-07	Q

Contig	BLAST result	e value	Class
contig07854	gi|156543794|ref|XP_001606411.1| PREDICTED: similar to Inositol ... 57	4E-07	I
contig09414	gi|67083845|gb|AAY66857.1| TATA box binding protein (TBP)-associ... 57	4E-07	I
contig01557	gi|158287182|ref|XP_560266.3| AGAP001039-PA [*Anopheles gambiae* s... 58	2E-07	E
contig03084	gi|72021459|ref|XP_797239.1| PREDICTED: similar to 3 beta-hydrox... 59	2E-07	IE
contig07860	gi|195397451|ref|XP_002057342.1| GJ16401 [*Drosophila virilis*] >g... 59	2E-07	I
contig08466	gi|193676359|ref|XP_001952377.1| PREDICTED: similar to GA19958-P... 58	2E-07	G
contig11296	gi|115639106|ref|XP_001200384.1| PREDICTED: hypothetical protein... 59	2E-07	C
contig12543	gi|17570195|ref|NP_508868.1| VITellogenin structural genes (yolk... 60	2E-07	H
contig04731	gi|193587209|ref|XP_001950230.1| PREDICTED: similar to NADH:ubiq... 48	1E-07	C
contig05107	gi|110762650|ref|XP_395129.3| PREDICTED: similar to CG10413-PA [... 59	1E-07	I
contig11211	gi|170050814|ref|XP_001861480.1| amino acid transporter [*Culex q...* 59	1E-07	C
contig08281	gi|193641036|ref|XP_001948702.1| PREDICTED: similar to LN1 [Acyr... 59	9E-08	E
contig09534	gi|156390511|ref|XP_001635314.1| predicted protein [*Nematostella*... 59	9E-08	C
contig03264	gi|39577847|gb|AAR28532.1| plasma membrane calcium ATPase PMCA3 ... 60	7E-08	C
contig08174	gi|66516122|ref|XP_393022.2| PREDICTED: similar to lethal (2) 44... 60	6E-08	G
contig09501	gi|187608290|ref|NP_001120172.1| hypothetical protein LOC1001452... 60	5E-08	I
contig06275	gi|6934272|gb|AAF31692.1|AF182744_1 Niemann-Pick type C1 protein... 60	4E-08	P
contig06498	gi|149048722|gb|EDM01263.1| acyl-Coenzyme A dehydrogenase family... 60	4E-08	C
contig05319	gi|193605977|ref|XP_001949683.1| PREDICTED: similar to AGAP00551... 61	3E-08	Q
contig11167	gi|195999632|ref|XP_002109684.1| hypothetical protein TRIADDRAFT... 61	3E-08	C
contig12071	gi|109118856|ref|XP_001109939.1| PREDICTED: acid alpha-glucosida... 61	3E-08	G
contig03564	gi|91781275|ref|YP_556482.1| putative ribokinase [*Burkholderia x*... 61	2E-08	C
contig05742	gi|6572446|emb|CAB63112.1| serine protease [*Pacifastacus leniusc*... 62	2E-08	C
contig08270	gi|17555004|ref|NP_498363.1| T12A2.1 [*Caenorhabditis elegans*] >g... 61	2E-08	E
contig10151	gi|118794464|ref|XP_321495.3| AGAP001606-PA [*Anopheles gambiae* s... 62	2E-08	I
contig11879	gi|156544090|ref|XP_001605489.1| PREDICTED: similar to bicaudal-... 61	2E-08	I
contig00278	gi|162287393|ref|NP_001104713.1| hypothetical protein LOC1000030... 63	1E-08	P
contig01488	gi|193210674|ref|NP_497437.2| Y22D7AL.14 [*Caenorhabditis elegans*] 62	1E-08	E
contig04660	gi|195122078|ref|XP_002005539.1| GI19014 [*Drosophila mojavensis*]... 62	1E-08	I
contig09965	gi|47211546|emb|CAF96111.1| unnamed protein product [*Tetraodon n*... 62	1E-08	P
contig12511	gi|158300633|ref|XP_552182.3| AGAP012028-PA [*Anopheles gambiae* s... 62	1E-08	G
contig06164	gi|110755389|ref|XP_001121254.1| PREDICTED: similar to CG11796-P... 63	7E-09	QI
contig05993	gi|66513180|ref|XP_623441.1| PREDICTED: similar to CG7712-PA [Ap... 63	6E-09	C
contig06881	gi|170032877|ref|XP_001844306.1| lysosomal acid lipase [*Culex qu*... 63	6E-09	I
contig11751	gi|195055751|ref|XP_001994776.1| GH17422 [*Drosophila grimshawi*] ... 63	6E-09	I
contig01134	gi|17570463|ref|NP_508505.1| Y71H10A.2 [*Caenorhabditis elegans*] ... 64	5E-09	G
contig01319	gi|67083923|gb|AAY66896.1| stunted-like [*Ixodes scapularis*] 64	5E-09	C
contig08920	gi|195442552|ref|XP_002069018.1| GK12296 [*Drosophila willistoni*]... 64	5E-09	P
contig00282	gi|18859837|ref|NP_573109.1| CG3415 CG3415-PA [*Drosophila melano*... 64	4E-09	E
contig00470	gi|156353921|ref|XP_001623156.1| predicted protein [*Nematostella*... 66	4E-09	I
contig03012	gi|193666972|ref|XP_001944972.1| PREDICTED: similar to AGAP00888... 64	4E-09	E
contig06071	gi|46318071|gb|AAS87603.1| cytochrome P450 CYP331A1 [*Capitella c*... 64	4E-09	G
contig05001	gi|149922588|ref|ZP_01911017.1| Acyl-CoA dehydrogenase [*Plesiocy*... 64	3E-09	E
contig05125	gi|149449616|ref|XP_001519228.1| PREDICTED: similar to ELOVL fam... 64	3E-09	G
contig08394	gi|193693010|ref|XP_001950596.1| PREDICTED: similar to zinc meta... 64	3E-09	F
contig01141	gi|170055692|ref|XP_001863695.1| neutral and basic amino acid tr... 67	2E-09	P
contig06262	gi|159464395|ref|XP_001690427.1| phosphoserine aminotransferase ... 65	2E-09	Q
contig07125	gi|110761779|ref|XP_397155.3| PREDICTED: similar to lethal (2) k... 65	2E-09	E
contig07128	gi|66547344|ref|XP_396740.2| PREDICTED: similar to CG12268-PA, i... 65	2E-09	E
contig07463	gi|195390815|ref|XP_002054063.1| GJ22998 [*Drosophila virilis*] >g... 65	2E-09	H
contig08791	gi|194226510|ref|XP_001490382.2| PREDICTED: similar to cytochrom... 65	2E-09	I

Contig	BLAST result	e value	Class
contig10011	gi|156360723|ref|XP_001625175.1| predicted protein [*Nematostella*... 65	2E-09	P
contig12054	gi|156408031|ref|XP_001641660.1| predicted protein [*Nematostella*... 54	2E-09	IQ
contig13235	gi|189233593|ref|XP_970417.2| PREDICTED: similar to fatty acid s... 60	2E-09	F
contig02737	gi|170062302|ref|XP_001866609.1| mitochondrial solute carrier pr... 66	1E-09	I
contig06613	gi|91082385|ref|XP_968820.1| PREDICTED: similar to alpha-tocophe... 65	1E-09	F
contig09114	gi|66516122|ref|XP_393022.2| PREDICTED: similar to lethal (2) 44... 65	1E-09	E
contig04249	gi|58384648|ref|XP_313383.2| AGAP003623-PA [*Anopheles gambiae* st... 66	8E-10	Q
contig04595	gi|91093016|ref|XP_969291.1| PREDICTED: similar to Glycerol kina... 66	8E-10	G
contig08760	gi|50289223|ref|XP_447042.1| unnamed protein product [*Candida gl*... 66	8E-10	I
contig10186	gi|158291516|ref|XP_313023.3| AGAP004142-PA [*Anopheles gambiae* s... 66	8E-10	EF
contig06429	gi|194217344|ref|XP_001501968.2| PREDICTED: similar to Solute ca... 67	6E-10	I
contig08439	gi|115738308|ref|XP_781908.2| PREDICTED: similar to Chain , Avi... 67	6E-10	G
contig05775	gi|54020920|ref|NP_001005704.1| enoyl Coenzyme A hydratase 1, pe... 67	5E-10	I
contig11604	gi|195391400|ref|XP_002054348.1| GJ24393 [*Drosophila virilis*] >g... 68	4E-10	G
contig01179	gi|193579990|ref|XP_001945355.1| PREDICTED: similar to GA10697-P... 68	3E-10	E
contig04519	gi|41054629|ref|NP_956848.1| methylthioadenosine phosphorylase [... 68	3E-10	G
contig04887	gi|156543509|ref|XP_001602197.1| PREDICTED: similar to GA10181-P... 68	3E-10	I
contig07721	gi|125853302|ref|XP_689157.2| PREDICTED: similar to Mtrr protein... 68	3E-10	G
contig02144	gi|164420731|ref|NP_001030360.2| transaldolase 1 [*Bos taurus*] 68	2E-10	I
contig06950	gi|68405171|ref|XP_686778.1| PREDICTED: similar to 5-nucleotidas... 68	2E-10	G
contig08591	gi|47523816|ref|NP_999545.1| 3-hydroxy-3-methylglutaryl-Coenzyme... 69	2E-10	H
contig02585	gi|156549646|ref|XP_001604406.1| PREDICTED: similar to GA13300-P... 47	1E-10	C
contig03961	gi|47224441|emb|CAG08691.1| unnamed protein product [*Tetraodon n*... 69	1E-10	I
contig03499	gi|115749010|ref|XP_785115.2| PREDICTED: hypothetical protein [*S*... 69	9E-11	E
contig09909	gi|198130674|gb|EDY67487.1| GA26865 [*Drosophila pseudoobscura* ps... 69	9E-11	C
contig13247	gi|156551075|ref|XP_001602774.1| PREDICTED: similar to ENSANGP00... 70	8E-11	P
contig05446	gi|37958173|gb|AAP35081.1| Der f Alt a 10 allergen [*Dermatophago*... 70	7E-11	C
contig05632	gi|47216717|emb|CAG00991.1| unnamed protein product [*Tetraodon n*... 70	7E-11	F
contig11760	gi|156537421|ref|XP_001606891.1| PREDICTED: similar to arsenical... 70	7E-11	G
contig03060	gi|145546067|ref|XP_001458717.1| hypothetical protein GSPATT0002... 50	6E-11	E
contig07122	gi|156539123|ref|XP_001599438.1| PREDICTED: similar to GA21592-P... 70	5E-11	I
contig03001	gi|195473339|ref|XP_002088953.1| GE18859 [*Drosophila yakuba*] >gn... 73	4E-11	P
contig05992	gi|194211027|ref|XP_001493619.2| PREDICTED: similar to AMP deami... 70	4E-11	P
contig06641	gi|171695246|ref|XP_001912547.1| unnamed protein product [*Podosp*... 70	4E-11	H
contig08694	gi|82658272|ref|NP_001032494.1| hypothetical protein LOC641476 [... 70	4E-11	G
contig09438	gi|126340819|ref|XP_001373749.1| PREDICTED: hypothetical protein... 70	4E-11	I
contig11236	gi|156545794|ref|XP_001605783.1| PREDICTED: similar to rCG31985 ... 70	4E-11	I
contig06230	gi|195382689|ref|XP_002050062.1| GJ21930 [*Drosophila virilis*] >g... 71	3E-11	P
contig07320	gi|2494071|sp|Q94688|ALDH9_POLMI Aldehyde dehydrogenase 9 (PM-AL... 71	3E-11	I
contig00974	gi|198150738|gb|EAL29865.2| GA12151 [Drosophila pseudoobscura ps... 72	2E-11	I
contig04655	gi|193587229|ref|XP_001951555.1| PREDICTED: similar to hydroxypy... 71	2E-11	G
contig04853	gi|74814733|sp|Q8I913.1|SMD13_LOXLA Sphingomyelin phosphodiester... 72	2E-11	F
contig06240	gi|197729548|ref|ZP_03182225.1| acyl-CoA synthetase [*Streptomyce*... 71	2E-11	Q
contig08014	gi|93277254|gb|ABF06444.1| electrogenic NBC-like protein [*Loligo*... 71	2E-11	Q
contig09800	gi|156377908|ref|XP_001630887.1| predicted protein [*Nematostella*... 71	2E-11	E
contig01404	gi|194384202|dbj|BAG64874.1| unnamed protein product [*Homo sapiens*] 72	1E-11	C
contig01852	gi|81158008|dbj|BAE48254.1| extracellular superoxide dismutase [... 72	1E-11	H
contig04996	gi|91093513|ref|XP_969441.1| PREDICTED: similar to tpr repeat nu... 72	1E-11	C
contig10052	gi|193575579|ref|XP_001951870.1| PREDICTED: similar to hemolymph... 72	1E-11	F
contig11378	gi|195387487|ref|XP_002052427.1| GJ21736 [*Drosophila virilis*] >g... 66	1E-11	E
contig11456	gi|193659702|ref|XP_001950963.1| PREDICTED: similar to glucose t... 72	1E-11	Q

Contig	BLAST result	e value	Class
contig07639	gi|50754481|ref|XP_414404.1| PREDICTED: similar to Pyruvate dehy... 73	8E-12	H
contig00480	gi|196013916|ref|XP_002116818.1| hypothetical protein TRIADDRAFT... 73	6E-12	C
contig03104	gi|145225845|ref|YP_001136523.1| AMP-dependent synthetase and li... 73	6E-12	C
contig03682	gi|195126086|ref|XP_002007505.1| GI12986 [*Drosophila mojavensis*]... 73	6E-12	F
contig02662	gi|195108859|ref|XP_001999010.1| GI24279 [*Drosophila mojavensis*]... 76	4E-12	C
contig05545	gi|195997975|ref|XP_002108856.1| hypothetical protein TRIADDRAFT... 74	4E-12	I
contig06536	gi|58387114|ref|XP_315337.2| AGAP005323-PB [*Anopheles gambiae* st... 75	4E-12	E
contig07942	gi|170065070|ref|XP_001867789.1| conserved hypothetical protein ... 74	4E-12	Q
contig08376	gi|167517935|ref|XP_001743308.1| predicted protein [*Monosiga bre*... 74	4E-12	E
contig08995	gi|157110002|ref|XP_001650914.1| carnitine o-acyltransferase [*Ae*... 74	4E-12	E
contig03072	gi|198137984|gb|EDY69913.1| GA25872 [Drosophila pseudoobscura ps... 74	3E-12	F
contig03825	gi|110762117|ref|XP_001122114.1| PREDICTED: similar to cysteine ... 74	3E-12	G
contig04073	gi|189240007|ref|XP_966693.2| PREDICTED: similar to vacuolar ATP... 74	3E-12	E
contig05414	gi|194863622|ref|XP_001970531.1| GG23324 [*Drosophila erecta*] >gn... 74	3E-12	E
contig10456	gi|39577847|gb|AAR28532.1| plasma membrane calcium ATPase PMCA3 ... 74	3E-12	C
contig10657	gi|195013559|ref|XP_001983861.1| GH16129 [*Drosophila grimshawi*] ... 74	3E-12	C
contig00113	gi|48101426|ref|XP_392672.1| PREDICTED: similar to CG1746-PA, is... 76	2E-12	G
contig03600	gi|157117432|ref|XP_001658764.1| tyrosine transporter [*Aedes aeg*... 75	2E-12	H
contig03644	gi|55736035|gb|AAV63544.1| fed tick salivary protein 10 [*Ixodes* ... 75	2E-12	E
contig04018	gi|158302403|ref|XP_321957.3| AGAP001200-PA [*Anopheles gambiae* s... 75	2E-12	I
contig05068	gi|47218158|emb|CAG10078.1| unnamed protein product [*Tetraodon n*... 75	2E-12	E
contig07457	gi|147905676|ref|NP_001088580.1| hypothetical protein LOC495458 ... 75	2E-12	E
contig09721	gi|195382312|ref|XP_002049874.1| GJ21833 [*Drosophila virilis*] >g... 75	2E-12	P
contig01320	gi|163939191|ref|YP_001644075.1| aldehyde dehydrogenase [*Bacillu*... 76	1E-12	C
contig05061	gi|195128215|ref|XP_002008560.1| GI11746 [*Drosophila mojavensis*]... 76	1E-12	FE
contig05313	gi|156550556|ref|XP_001603294.1| PREDICTED: similar to ENSANGP00... 75	1E-12	Q
contig10868	gi|160373115|gb|ABX38842.1| multidrug resistance-associated prot... 58	1E-12	C
contig07263	gi|164425151|ref|XP_962376.2| hypothetical protein NCU06342 [*Neu*... 76	8E-13	E
contig01746	gi|56710319|ref|NP_001008665.1| platelet coagulation factor XI [... 52	7E-13	I
contig06907	gi|166158220|ref|NP_001107301.1| hypothetical protein LOC1001350... 76	7E-13	C
contig09186	gi|189239301|ref|XP_971644.2| PREDICTED: similar to utp-glucose-... 76	7E-13	G
contig09421	gi|47227107|emb|CAG00469.1| unnamed protein product [*Tetraodon n*... 76	7E-13	PQ
contig01459	gi|170040775|ref|XP_001848163.1| 4-nitrophenylphosphatase [*Culex*... 77	6E-13	C
contig04221	gi|156548821|ref|XP_001605394.1| PREDICTED: similar to CG9977-PA... 70	5E-13	C
contig08282	gi|55742254|ref|NP_001006720.1| nucleoside phosphorylase [*Xenopu*... 77	4E-13	G
contig09051	gi|189234287|ref|XP_970030.2| PREDICTED: similar to isocitrate d... 77	4E-13	Q
contig05744	gi|72088537|ref|XP_780121.1| PREDICTED: similar to cytosolic NAD... 77	3E-13	H
contig09205	gi|196476813|gb|ACG76270.1| adenosine kinase-like protein [*Ambly*... 77	3E-13	I
contig09573	gi|195028104|ref|XP_001986919.1| GH20262 [Drosophila grimshawi] ... 56	3E-13	P
contig10503	gi|158294869|ref|XP_315870.4| AGAP005845-PA [*Anopheles gambiae* s... 78	3E-13	QI
contig00622	gi|157773171|ref|XP_001664650.1| hypothetical protein CBG22471 [... 78	2E-13	E
contig02273	gi|158303308|ref|NP_062203.1| propionyl-coenzyme A carboxylase, ... 79	2E-13	I
contig03907	gi|198130649|gb|EDY67478.1| GA26930 [*Drosophila pseudoobscura* ps... 79	2E-13	F
contig04042	gi|110749750|ref|XP_624152.2| PREDICTED: similar to CG9977-PA [A... 78	2E-13	E
contig04558	gi|5921919|sp|O42563|CP3AR_ONCMY Cytochrome P450 3A27 (CYPIIIA27... 78	2E-13	C
contig04722	gi|115758805|ref|XP_001203713.1| PREDICTED: similar to cytochrom... 66	2E-13	P
contig09379	gi|57098467|ref|XP_540100.1| PREDICTED: similar to CG9413-PA, is... 78	2E-13	Q
contig10124	gi|115722514|ref|XP_001198640.1| PREDICTED: similar to CG9990-PB... 78	2E-13	P
contig12434	gi|170059729|ref|XP_001865488.1| NADH:ubiquinone dehydrogenase [... 79	2E-13	I
contig05650	gi|148224038|ref|NP_001089730.1| hypothetical protein LOC734793 ... 79	1E-13	F
contig07306	gi|161088656|gb|ABX57387.1| isocitrate dehydrogenase [*Kallima pa*... 79	1E-13	Q

Contig	BLAST result	e value	Class
contig09650	gi|156407402|ref|XP_001641533.1| predicted protein [*Nematostella*... 72	1E-13	C
contig10145	gi|126311408|ref|XP_001381865.1| PREDICTED: similar to glycogen ... 79	1E-13	E
contig10146	gi|91094733|ref|XP_975938.1| PREDICTED: similar to AGAP002830-PA... 79	1E-13	I
contig06732	gi|160872768|ref|ZP_02062900.1| fructose-bisphosphate aldolase c... 79	9E-14	PQ
contig08767	gi|193659690|ref|XP_001950251.1| PREDICTED: similar to AGAP00764... 79	9E-14	E
contig02949	gi|195575821|ref|XP_002077775.1| GD23108 [*Drosophila simulans*] >... 80	7E-14	I
contig04506	gi|195436172|ref|XP_002066043.1| GK22142 [*Drosophila willistoni*]... 80	5E-14	I
contig00091	gi|51011612|gb|AAT92215.1| cytochrome oxidase subunit VIIc [Ixod... 82	4E-14	G
contig04436	gi|156542993|ref|XP_001603137.1| PREDICTED: similar to ENSANGP00... 80	4E-14	I
contig11736	gi|170744118|ref|YP_001772773.1| phosphoribosylamine--glycine li... 80	4E-14	G
contig06032	gi|194219975|ref|XP_001504536.2| PREDICTED: similar to Alpha-ami... 81	3E-14	F
contig03968	gi|48101936|ref|XP_392725.1| PREDICTED: similar to CG18815-PA, i... 82	2E-14	P
contig05891	gi|195997821|ref|XP_002108779.1| hypothetical protein TRIADDRAFT... 82	2E-14	IE
contig06070	gi|166979753|sp|Q8BX37.2|PAPL_MOUSE Iron/zinc purple acid phosph... 82	2E-14	E
contig06084	gi|194383054|dbj|BAG59083.1| unnamed protein product [*Homo sapiens*] 81	2E-14	C
contig09842	gi|91082037|ref|XP_970725.1| PREDICTED: similar to AGAP004744-PA... 81	2E-14	G
contig02219	gi|157136674|ref|XP_001663817.1| sugar transporter [*Aedes aegypt*... 82	1E-14	E
contig03920	gi|193809405|emb|CAQ40107.1| aquaglyceroporin, putative [*Plasmod*... 67	1E-14	I
contig08867	gi|91091770|ref|XP_966678.1| PREDICTED: similar to S-adenosylmet... 82	1E-14	I
contig09745	gi|50344854|ref|NP_001002099.1| hypothetical protein LOC415189 [... 82	1E-14	Q
contig10706	gi|195570253|ref|XP_002103123.1| GD20259 [*Drosophila simulans*] >... 82	1E-14	G
contig03590	gi|56118640|ref|NP_001008120.1| pigm protein [*Xenopus tropicalis*... 83	8E-15	F
contig06694	gi|115772601|ref|XP_001183409.1| PREDICTED: hypothetical protein... 83	8E-15	P
contig07410	gi|212491|gb|AAA48996.1| ORF 83	8E-15	E
contig00348	gi|149699396|ref|XP_001501853.1| PREDICTED: similar to WW domain... 83	6E-15	G
contig06463	gi|193613296|ref|XP_001946535.1| PREDICTED: similar to CG6950 CG... 77	6E-15	C
contig02132	gi|42491539|gb|AAS16911.1| 71 kDa salivary protein [*Lutzomyia lo*... 55	5E-15	C
contig03778	gi|189546543|ref|XP_001919592.1| PREDICTED: similar to Solute ca... 84	5E-15	I
contig05228	gi|167522755|ref|XP_001745715.1| predicted protein [*Monosiga bre*... 84	5E-15	F
contig05680	gi|189537626|ref|XP_684074.3| PREDICTED: aminoadipate-semialdehy... 84	5E-15	G
contig10258	gi|115911562|ref|XP_793074.2| PREDICTED: hypothetical protein [*S*... 84	5E-15	C
contig04532	gi|193587221|ref|XP_001951102.1| PREDICTED: similar to lethal (2... 84	4E-15	E
contig08890	gi|154300406|ref|XP_001550619.1| hypothetical protein BC1G_11392... 77	4E-15	P
contig09427	gi|189238926|ref|XP_001811313.1| PREDICTED: similar to CG8814 CG... 84	4E-15	E
contig00934	gi|67083961|gb|AAY66915.1| ubiquinol-cytochrome C reductase comp... 86	3E-15	G
contig02494	gi|170032335|ref|XP_001844037.1| vacuolar ATP synthase subunit H... 84	3E-15	G
contig02846	gi|115666557|ref|XP_797442.2| PREDICTED: similar to Ppox-prov pr... 84	3E-15	I
contig01884	gi|187829489|ref|NP_001120706.1| ornithine decarboxylase antizym... 87	2E-15	H
contig03757	gi|194760099|ref|XP_001962279.1| GF15389 [*Drosophila ananassae*] ... 85	2E-15	Q
contig04496	gi|195426310|ref|XP_002061281.1| GK20832 [*Drosophila willistoni*]... 85	2E-15	G
contig08369	gi|156717370|ref|NP_001096225.1| SEC14-like 1 [*Xenopus* (*Silurana*... 85	2E-15	C
contig10349	gi|198131524|gb|EAL27542.2| GA21221 [*Drosophila pseudoobscura* ps... 85	2E-15	E
contig05570	gi|194862154|ref|XP_001969934.1| GG23666 [*Drosophila erecta*] >gn... 86	1E-15	P
contig09242	gi|194226528|ref|XP_001491279.2| PREDICTED: similar to ADP/ATP t... 86	1E-15	E
contig09484	gi|71895769|ref|NP_001026203.1| ChaC, cation transport regulator... 86	1E-15	C
contig01603	gi|73982759|ref|XP_864136.1| PREDICTED: similar to NADH-ubiquino... 86	9E-16	I
contig01880	gi|195431523|ref|XP_002063787.1| GK15853 [*Drosophila willistoni*]... 86	9E-16	I
contig05029	gi|156354255|ref|XP_001623314.1| predicted protein [*Nematostella*... 86	9E-16	F
contig01421	gi|193078240|gb|ABO13201.2| putative metallopeptidase [*Acinetoba*... 86	7E-16	C
contig02658	gi|195494019|ref|XP_002094662.1| GE20080 [*Drosophila yakuba*] >gn... 87	5E-16	P
contig04077	gi|149642867|ref|NP_001092520.1| elongation of very long chain f... 87	5E-16	I

Contig	BLAST result	e value	Class
contig10567	gi|170068294|ref|XP_001868812.1| glutamine-dependent NAD(+) synt... 87	5E-16	C
contig00857	gi|149618312|ref|XP_001510407.1| PREDICTED: similar to cytochrom... 89	4E-16	F
contig03083	gi|66547760|ref|XP_392684.2| PREDICTED: similar to Dihydropterid... 87	4E-16	F
contig06223	gi|1839194|gb|AAC47636.1| enolase [Dugesia cf. dorotocephala] 87	3E-16	C
contig10562	gi|195122754|ref|XP_002005876.1| GI20717 [*Drosophila mojavensis*]... 82	3E-16	C
contig00880	gi|91091346|ref|XP_972193.1| PREDICTED: similar to predicted pro... 88	2E-16	F
contig11097	gi|148231141|ref|NP_001083508.1| hypothetical protein LOC398964 ... 89	2E-16	I
contig11920	gi|156554132|ref|XP_001604231.1| PREDICTED: similar to ubiquinol... 88	2E-16	C
contig09092	gi|195148434|ref|XP_002015179.1| GL18556 [*Drosophila persimilis*]... 90	1E-16	G
contig01481	gi|194224697|ref|XP_001495027.2| PREDICTED: similar to fructose-... 89	9E-17	Q
contig04550	gi|189241870|ref|XP_971717.2| PREDICTED: similar to saccharopine... 55	9E-17	I
contig10515	gi|195033324|ref|XP_001988662.1| GH11286 [*Drosophila grimshawi*] ... 89	9E-17	F
contig09821	gi|170039628|ref|XP_001847631.1| acyl-CoA oxidase [*Culex quinque*... 89	8E-17	H
contig02270	gi|189525113|ref|XP_695050.3| PREDICTED: sulfite oxidase [*Danio* ... 90	7E-17	I
contig02795	gi|66517758|ref|XP_623474.1| PREDICTED: similar to Carbonyl redu... 91	6E-17	Q
contig03175	gi|194745602|ref|XP_001955276.1| GF18677 [*Drosophila ananassae*] ... 91	3E-17	E
contig11436	gi|77461455|ref|YP_350962.1| gamma-butyrobetaine hydroxylase [*Ps*... 92	3E-17	E
contig04721	gi|198151525|gb|EAL30569.2| GA19849 [*Drosophila pseudoobscura* ps... 91	2E-17	P
contig06116	gi|197245693|gb|AAI68641.1| Unknown (protein for MGC:186503) [*Xe*... 92	2E-17	F
contig06900	gi|149706173|ref|XP_001502362.1| PREDICTED: similar to NADH dehy... 92	2E-17	P
contig07626	gi|148235999|ref|NP_001085057.1| hypothetical protein LOC432127 ... 91	2E-17	F
contig03079	gi|156553614|ref|XP_001599996.1| PREDICTED: similar to CG31522-P... 92	1E-17	G
contig07804	gi|91084189|ref|XP_967176.1| PREDICTED: similar to AGAP002647-PA... 92	1E-17	E
contig08750	gi|124295296|gb|ABN04118.1| ATP/ADP translocase [*Marsupenaeus j*a... 92	1E-17	I
contig08899	gi|193662234|ref|XP_001948711.1| PREDICTED: similar to alanine a... 92	1E-17	HE
contig09038	gi|170057714|ref|XP_001864604.1| conserved hypothetical protein ... 67	1E-17	I
contig02410	gi|17507759|ref|NP_491464.1| F57C9.1b [*Caenorhabditis elegans*] >... 93	8E-18	E
contig03752	gi|55736029|gb|AAV63541.1| fed tick salivary protein 7 [*Ixodes s*... 94	8E-18	GC
contig04868	gi|195972843|ref|NP_001124421.1| hypothetical protein LOC1001748... 93	8E-18	I
contig06179	gi|163915469|gb|AAI57302.1| LOC548390 protein [*Xenopus tropicalis*] 93	6E-18	C
contig09855	gi|148230290|ref|NP_001080419.1| solute carrier family 25 (carni... 93	6E-18	F
contig06919	gi|16197889|gb|AAL13694.1| GH26644p [*Drosophila melanogaster*] 94	4E-18	P
contig09825	gi|158286795|ref|XP_308929.4| AGAP006817-PA [*Anopheles gambiae* s... 94	4E-18	C
contig01200	gi|66515272|ref|XP_623495.1| PREDICTED: similar to Vacuolar ATP ... 78	3E-18	I
contig07706	gi|115720214|ref|XP_789770.2| PREDICTED: similar to aquaporin 9 ... 94	3E-18	P
contig08050	gi|195058786|ref|XP_001995500.1| GH17781 [*Drosophila grimshawi*] ... 94	3E-18	E
contig08919	gi|195503375|ref|XP_002098625.1| GE23836 [*Drosophila yakuba*] >gn... 94	3E-18	I
contig11615	gi|195474885|ref|XP_002089720.1| GE22674 [*Drosophila yakuba*] >gn... 94	3E-18	E
contig12284	gi|119352607|gb|ABL63906.1| Na,K,Cl-cotransporter [*Amblyomma a*me... 94	3E-18	E
contig13294	gi|195954016|ref|YP_002122317.1| cytochrome c oxidase subunit II... 94	3E-18	P
contig00011	gi|32481105|gb|AAP83944.1| ATPase subunit 6 [*Rhipicephalus haema*... 96	2E-18	C
contig00651	gi|194332534|ref|NP_001123756.1| hypothetical protein LOC1001705... 75	2E-18	C
contig01773	gi|195124843|ref|XP_002006893.1| GI21317 [*Drosophila mojavensis*]... 96	2E-18	I
contig02575	gi|119925522|ref|XP_001253431.1| PREDICTED: similar to Monoacylg... 95	2E-18	C
contig02857	gi|158301606|ref|XP_321277.4| AGAP001791-PA [*Anopheles gambiae* s... 95	2E-18	G
contig03918	gi|26337211|dbj|BAC32290.1| unnamed protein product [*Mus musculus*] 95	2E-18	F
contig04691	gi|194226282|ref|XP_001491158.2| PREDICTED: similar to ECHOS1 [E... 95	2E-18	RP
contig04973	gi|14485281|ref|NP_058968.14| aldehyde dehydrogenase family 1, s... 95	2E-18	I
contig08359	gi|65332146|gb|AAY42207.1| short chain dehydrogenase-like protei... 58	2E-18	E
contig00295	gi|166219081|gb|ABY85409.1| chitinase [*Scylla serrata*] 96	1E-18	Q
contig09621	gi|156384751|ref|XP_001633296.1| predicted protein [*Nematostella*... 96	1E-18	I

Contig	BLAST result	e value	Class
contig00709	gi|6179898|gb|AAF05702.1|AF190129_1 Na+/K+/2Cl- cotransporter [*C*... 96	9E-19	G
contig02844	gi|38570373|gb|AAR24625.1| tryptophan oxygenase [*Plodia interpun*... 96	9E-19	Q
contig04483	gi|74096077|ref|NP_001027723.1| ATP sulfurylase/APS kinase [*Cion*... 96	9E-19	C
contig04999	gi|156547798|ref|XP_001606202.1| PREDICTED: similar to organic a... 96	9E-19	C
contig01778	gi|158293496|ref|XP_314839.4| AGAP008727-PA [*Anopheles gambiae* s... 98	8E-19	G
contig01055	gi|156380971|ref|XP_001632040.1| predicted protein [*Nematostella*... 97	5E-19	P
contig10742	gi|195389915|ref|XP_002053617.1| GJ23253 [*Drosophila virilis*] >g... 97	5E-19	I
contig12600	gi|195328533|ref|XP_002030969.1| GM24286 [*Drosophila sechellia*] ... 98	5E-19	P
contig01342	gi|66553147|ref|XP_624346.1| PREDICTED: similar to Vacuolar ATP ... 97	3E-19	C
contig01037	gi|76156087|gb|AAX27321.2| SJCHGC02398 protein [*Schistosoma japo*... 98	2E-19	H
contig04609	gi|47218833|emb|CAG02818.1| unnamed protein product [*Tetraodon n*... 99	1E-19	Q
contig07380	gi|196002753|ref|XP_002111244.1| hypothetical protein TRIADDRAFT... 99	1E-19	E
contig03334	gi|157132079|ref|XP_001662452.1| coupling factor, putative [*Aede*... 99	8E-20	P
contig04559	gi|118794426|ref|XP_321470.3| AGAP001627-PA [*Anopheles gambiae* s... 85	8E-20	C
contig08673	gi|126320626|ref|XP_001368242.1| PREDICTED: similar to Chain B, ... 99	8E-20	F
contig01291	gi|156553749|ref|XP_001601392.1| PREDICTED: similar to gamma-glu... 89	7E-20	Q
contig01730	gi|91091864|ref|XP_968949.1| PREDICTED: similar to NADH-ubiquino... 101	6E-20	F
contig02848	gi|12644305|sp|P52307.2|5NTD_BOOMI Protein 5NUC precursor [Inclu... 100	6E-20	C
contig08379	gi|157132046|ref|XP_001662436.1| potassium/chloride symporter, p... 100	6E-20	PQ
contig03126	gi|156408908|ref|XP_001642098.1| predicted protein [*Nematostella*... 101	4E-20	H
contig11494	gi|91088121|ref|XP_970262.1| PREDICTED: similar to nudix hydrola... 100	4E-20	E
contig03553	gi|157134166|ref|XP_001656319.1| glycine cleavage system h prote... 66	3E-20	I
contig05716	gi|189237128|ref|XP_972994.2| PREDICTED: similar to sodium bicar... 100	3E-20	G
contig01355	gi|156408582|ref|XP_001641935.1| predicted protein [*Nematostella*... 101	2E-20	C
contig02484	gi|193636681|ref|XP_001947434.1| PREDICTED: similar to multidrug... 101	2E-20	C
contig01125	gi|156555239|ref|XP_001604130.1| PREDICTED: similar to MGC52653 ... 102	1E-20	I
contig02714	gi|156555760|ref|XP_001602610.1| PREDICTED: similar to CG7430-PA... 102	1E-20	IG
contig02970	gi|70887737|ref|NP_001020716.1| hypothetical protein LOC566769 [... 102	1E-20	P
contig06193	gi|110764096|ref|XP_624260.2| PREDICTED: similar to hoepel1 CG12... 102	1E-20	C
contig07567	gi|91076040|ref|XP_972204.1| PREDICTED: similar to CG1814 CG1814... 102	1E-20	I
contig11077	gi|26000552|gb|AAN75454.1| Na-dependent Cl/HCO3 exchanger [*Lolig*... 102	1E-20	I
contig04710	gi|156543124|ref|XP_001605583.1| PREDICTED: similar to tpr repea... 102	7E-21	C
contig05417	gi|47086263|ref|NP_998051.1| phosphoglucomutase 2 [*Danio rerio*] ... 86	7E-21	C
contig05671	gi|193659714|ref|XP_001943295.1| PREDICTED: similar to AGAP00609... 73	7E-21	C
contig10003	gi|26420454|gb|AAN78224.1| factor D-like protein [*Dermacentor* va... 102	7E-21	C
contig00405	gi|149721010|ref|XP_001492275.1| PREDICTED: similar to Thymidyla... 103	6E-21	P
contig00046	gi|74186440|dbj|BAE42978.1| unnamed protein product [*Mus musculus*] 105	4E-21	I
contig04277	gi|88853843|ref|NP_001034691.1| solute carrier family 27, member... 62	4E-21	Q
contig12094	gi|33286942|gb|AAH55383.1| Echs1 protein [*Danio rerio*] 103	4E-21	C
contig06733	gi|94692224|gb|ABF46831.1| multidrug resistance protein MRP2 [*Ma*... 104	3E-21	I
contig07465	gi|170049513|ref|XP_001857207.1| serine protease [*Culex quinquef*... 103	3E-21	C
contig10702	gi|91076782|ref|XP_967792.1| PREDICTED: similar to sphingosine p... 104	3E-21	I
contig11252	gi|69933293|ref|ZP_00628495.1| Deoxyribose-phosphate aldolase [*P*... 104	3E-21	G
contig06884	gi|36938566|gb|AAQ86831.1| chloride channel [*Ixodes scapularis*] 104	2E-21	C
contig05402	gi|195503929|ref|XP_002098862.1| GE10606 [*Drosophila yakuba*] >gn... 105	1E-21	EF
contig05512	gi|49474815|ref|YP_032857.1| succinyl-CoA synthetase subunit alp... 72	1E-21	E
contig01770	gi|189240738|ref|XP_968166.2| PREDICTED: similar to AGAP007574-P... 107	9E-22	G
contig03137	gi|195583780|ref|XP_002081694.1| GD25577 [*Drosophila simulans*] >... 105	9E-22	C
contig01727	gi|195380171|ref|XP_002048844.1| GJ21093 [*Drosophila virilis*] >g... 73	7E-22	C
contig04271	gi|187026108|emb|CAP34759.2| C. briggsae CBR-TAT-5 protein [*Caen*... 106	7E-22	F
contig06843	gi|189238261|ref|XP_974441.2| PREDICTED: similar to abc transpor... 106	5E-22	G

Contig	BLAST result	e value	Class
contig02919	gi|147904583|ref|NP_001090373.1| electron transfer flavoprotein,... 107	4E-22	C
contig03185	gi|47217590|emb|CAG02517.1| unnamed protein product [*Tetraodon n*... 107	4E-22	Q
contig05160	gi|169768148|ref|XP_001818545.1| hypothetical protein [*Aspergill*... 107	4E-22	E
contig06718	gi|156547213|ref|XP_001604483.1| PREDICTED: similar to homogenti... 107	3E-22	I
contig08291	gi|158286683|ref|XP_308864.3| AGAP006891-PA [*Anopheles gambiae* s... 107	3E-22	E
contig01710	gi|156405693|ref|XP_001640866.1| predicted protein [*Nematostella*... 107	2E-22	G
contig01745	gi|195113251|ref|XP_002001181.1| GI10642 [*Drosophila mojavensis*]... 108	2E-22	C
contig03910	gi|91094297|ref|XP_971720.1| PREDICTED: similar to steroid dehyd... 108	2E-22	I
contig01873	gi|189237972|ref|XP_001812042.1| PREDICTED: similar to AGAP00927... 108	1E-22	E
contig03288	gi|66533950|ref|XP_623800.1| PREDICTED: similar to Succinyl coen... 108	1E-22	G
contig03345	gi|195429930|ref|XP_002063010.1| GK21608 [*Drosophila willistoni*]... 108	1E-22	G
contig04746	gi|156546442|ref|XP_001607215.1| PREDICTED: similar to ENSANGP00... 108	1E-22	C
contig06406	gi|149023839|gb|EDL80336.1| rCG30713, isoform CRA_a [*Rattus norv*... 108	1E-22	F
contig07331	gi|109097119|ref|XP_001098147.1| PREDICTED: similar to citrate s... 108	1E-22	G
contig02312	gi|50753071|ref|XP_413855.1| PREDICTED: similar to Fumarylacetoa... 109	8E-23	F
contig06138	gi|115440181|ref|NP_001044370.1| Os01g0769100 [*Oryza sativa* (*jap*... 109	8E-23	C
contig02694	gi|195454346|ref|XP_002074200.1| GK12747 [*Drosophila willistoni*]... 110	5E-23	P
contig11269	gi|17561328|ref|NP_506376.1| F53F4.10 [*Caenorhabditis elegans*] >... 110	5E-23	C
contig08317	gi|170095097|ref|XP_001878769.1| predicted protein [*Laccaria bic*... 110	4E-23	H
contig06551	gi|47217700|emb|CAG13331.1| unnamed protein product [*Tetraodon n*... 110	2E-23	C
contig06753	gi|157105391|ref|XP_001648848.1| ATP-citrate synthase [*Aedes aeg*... 111	2E-23	P
contig08052	gi|193215860|ref|YP_001997059.1| isocitrate dehydrogenase, NAD-d... 111	2E-23	I
contig11019	gi|156547848|ref|XP_001604320.1| PREDICTED: similar to ENSANGP00... 111	2E-23	G
contig05000	gi|195052348|ref|XP_001993284.1| GH13153 [*Drosophila grimshawi*] ... 91	1E-23	E
contig05174	gi|113681577|ref|NP_001038619.1| hypothetical protein LOC568160 ... 112	1E-23	I
contig07679	gi|157113072|ref|XP_001651879.1| acetyl-coa carboxylase [*Aedes a*... 112	1E-23	G
contig08352	gi|157110450|ref|XP_001651107.1| plasma membrane calcium-transpo... 112	1E-23	C
contig05215	gi|148227451|ref|NP_001089298.1| hypothetical protein LOC734347 ... 112	7E-24	F
contig01305	gi|91090998|ref|XP_974932.1| PREDICTED: similar to AGAP003680-PA... 113	6E-24	I
contig10588	gi|71896949|ref|NP_001026492.1| major facilitator superfamily do... 113	4E-24	F
contig12295	gi|115741846|ref|XP_001198932.1| PREDICTED: similar to CG9990-PB... 96	4E-24	E
contig03332	gi|62955483|ref|NP_001017755.1| NADH dehydrogenase (ubiquinone) ... 114	2E-24	FQ
contig07351	gi|108744354|gb|ABG02425.1| glutathione reductase [*Tigriopus jap*... 114	2E-24	I
contig08142	gi|66531425|ref|XP_623450.1| PREDICTED: similar to CG7920-PA, is... 114	2E-24	F
contig08770	gi|156390853|ref|XP_001635484.1| predicted protein [*Nematostella*... 114	2E-24	E
contig10437	gi|195486942|ref|XP_002091716.1| GE12096 [*Drosophila yakuba*] >gn... 114	2E-24	C
contig01321	gi|109128739|ref|XP_001103601.1| PREDICTED: aspartate aminotrans... 115	1E-24	E
contig12876	gi|157929880|gb|ABW04128.1| endozepine [*Epinephelus coioides*] 116	6E-25	PQ
contig05017	gi|152207621|gb|ABS30897.1| 5'-nucleotidase/putative apyrase iso... 116	5E-25	C
contig05648	gi|60302696|ref|NP_001012540.1| ATP-binding cassette, sub-family... 116	5E-25	C
contig11305	gi|170582233|ref|XP_001896037.1| N-myristoyltransferase 2 [*Brugi*... 116	5E-25	P
contig00940	gi|50752839|ref|XP_413770.1| PREDICTED: hypothetical protein [*Ga*... 117	4E-25	I
contig04449	gi|170034336|ref|XP_001845030.1| isocitrate dehydrogenase [*Culex*... 117	4E-25	I
contig02899	gi|53933295|ref|NP_990229.1| thymidine kinase 1, soluble [*Gallus*... 117	3E-25	H
contig04330	gi|115640689|ref|XP_780760.2| PREDICTED: hypothetical protein [*S*... 117	3E-25	C
contig02788	gi|157114716|ref|XP_001652386.1| methylmalonate-semialdehyde deh... 118	2E-25	E
contig03235	gi|114707413|ref|ZP_01440309.1| probable enolase protein [*Fulvim*... 117	2E-25	P
contig04150	gi|195392792|ref|XP_002055038.1| GJ19157 [*Drosophila virilis*] >g... 117	2E-25	E
contig01676	gi|62857761|ref|NP_001017234.1| steroid dehydrogenase-like [*Xeno*... 121	1E-25	P
contig10898	gi|193783653|dbj|BAG53564.1| unnamed protein product [*Homo sapiens*] 119	1E-25	I
contig03902	gi|115733116|ref|XP_787188.2| PREDICTED: similar to L-3-hydroxya... 119	9E-26	I

Contig	BLAST result	e value	Class
contig11001	gi|170054407|ref|XP_001863115.1| cytosolic purine 5-nucleotidase... 119	6E-26	I
contig02329	gi|72042689|ref|XP_794417.1| PREDICTED: similar to Retinol dehyd... 120	5E-26	H
contig03180	gi|156547719|ref|XP_001605273.1| PREDICTED: similar to MGC97830 ... 120	5E-26	G
contig02302	gi|45382681|ref|NP_990806.1| Na,K-ATPase alpha-3-subunit [*Gallus*... 120	4E-26	C
contig07528	gi|38194439|gb|AAR13229.1| glycerol-3-phosphate dehydrogenase [*C*... 115	4E-26	I
contig00584	gi|156396842|ref|XP_001637601.1| predicted protein [*Nematostella*... 120	3E-26	Q
contig05945	gi|194034149|ref|XP_001924714.1| PREDICTED: similar to adenylate... 120	3E-26	P
contig11030	gi|156551900|ref|XP_001606799.1| PREDICTED: similar to ENSANGP00... 121	2E-26	QI
contig12273	gi|67084103|gb|AAY66986.1| F1F0-type ATP synthase subunit g [Ixo... 121	2E-26	I
contig13318	gi|156548537|ref|XP_001602077.1| PREDICTED: similar to ENSANGP00... 121	2E-26	G
contig04932	gi|156393904|ref|XP_001636567.1| predicted protein [*Nematostella*... 113	6E-27	G
contig01284	gi|45219771|gb|AAH66902.1| Myo-inositol 1-phosphate synthase A1 ... 108	2E-27	I
contig01688	gi|115942978|ref|XP_787078.2| PREDICTED: hypothetical protein [*S*... 108	2E-27	G
contig07429	gi|156542905|ref|XP_001601219.1| PREDICTED: similar to adenosine... 126	2E-27	E
contig09760	gi|157813368|gb|ABV81429.1| putative hypothetical 44.9 kDa prote... 125	1E-27	C
contig07527	gi|157776283|ref|XP_001666207.1| hypothetical protein CBG09180 [... 126	6E-28	C
contig00596	gi|186920389|gb|ACC95446.1| glutamate dehydrogenase [*Litopenaeus*... 112	5E-28	C
contig05404	gi|156551734|ref|XP_001599719.1| PREDICTED: similar to conserved... 126	5E-28	Q
contig07048	gi|193591861|ref|XP_001945864.1| PREDICTED: similar to serine pr... 127	4E-28	G
contig00009	gi|156547609|ref|XP_001603441.1| PREDICTED: similar to CG8680-PA... 128	3E-28	I
contig02821	gi|157110519|ref|XP_001651137.1| utp-glucose-1-phosphate uridyly... 128	2E-28	F
contig01306	gi|23308625|ref|NP_694493.1| fatty acid binding protein 3, muscl... 130	1E-28	P
contig01538	gi|47522760|ref|NP_999131.1| long-chain 3-ketoacyl-CoA thiolase ... 129	1E-28	QI
contig03485	gi|195126843|ref|XP_002007878.1| GI13181 [*Drosophila mojavensis*]... 128	1E-28	C
contig08289	gi|91084231|ref|XP_969408.1| PREDICTED: similar to pyrroline-5-c... 128	1E-28	I
contig00926	gi|110757125|ref|XP_625036.2| PREDICTED: similar to Phosphoribos... 129	7E-29	H
contig08152	gi|156406811|ref|XP_001641238.1| predicted protein [*Nematostella*... 129	7E-29	G
contig00981	gi|94421562|gb|ABF18888.1| delta-9 desaturase [*Lygus lineolaris*] 131	4E-29	G
contig02432	gi|194881310|ref|XP_001974791.1| GG21959 [*Drosophila erecta*] >gn... 130	3E-29	C
contig04152	gi|156548342|ref|XP_001603387.1| PREDICTED: similar to CG3297-PB... 130	3E-29	E
contig10378	gi|189095482|ref|YP_001936641.1| NADH dehydrogenase subunit 4 [*L*... 130	3E-29	C
contig04130	gi|158288815|ref|XP_310644.4| AGAP000448-PA [*Anopheles gambiae* s... 131	2E-29	C
contig05489	gi|47199333|emb|CAF87694.1| unnamed protein product [*Tetraodon n*... 131	2E-29	G
contig12884	gi|30102420|gb|AAP03985.1| cytochrome c oxidase subunit 1 [*Strat*... 131	2E-29	C
contig06384	gi|156390853|ref|XP_001635484.1| predicted protein [*Nematostella*... 132	1E-29	F
contig02590	gi|51949802|gb|AAU14833.1| adenosine kinase isoform 2S [*Nicotian*... 132	7E-30	E
contig07069	gi|66511322|ref|XP_623076.1| PREDICTED: similar to CG9520-PB, is... 132	7E-30	I
contig11343	gi|74003677|ref|XP_545253.2| PREDICTED: similar to major facilit... 134	7E-30	G
contig11909	gi|67083995|gb|AAY66932.1| cytochrome c oxidase polypeptide Vb [... 135	7E-30	E
contig07666	gi|198151502|gb|EAL30537.2| GA13420 [*Drosophila pseudoobscura* ps... 133	5E-30	P
contig01696	gi|31202411|ref|XP_310154.1| AGAP009537-PA [*Anopheles gambiae* st... 133	4E-30	E
contig03525	gi|91082435|ref|XP_970856.1| PREDICTED: similar to AGAP010039-PA... 94	4E-30	C
contig12978	gi|3970893|dbj|BAA34806.1| serin proteinase 2 [*Haemaphysalis lon*... 135	3E-30	I
contig05815	gi|167519412|ref|XP_001744046.1| predicted protein [*Monosiga bre*... 134	2E-30	I
contig05937	gi|195131189|ref|XP_002010033.1| GI14910 [*Drosophila mojavensis*]... 134	2E-30	I
contig05468	gi|147906027|ref|NP_001088098.1| hypothetical protein LOC494796 ... 135	1E-30	G
contig10510	gi|47218252|emb|CAF96289.1| unnamed protein product [*Tetraodon n*... 135	1E-30	G
contig11535	gi|27311161|ref|NP_758881.1| NADH dehydrogenase subunit 4 [*Varro*... 139	5E-31	C
contig01308	gi|195578891|ref|XP_002079297.1| GD23872 [*Drosophila simulans*] >... 138	2E-31	G
contig02961	gi|156542476|ref|XP_001599672.1| PREDICTED: similar to ENSANGP00... 137	2E-31	I
contig07844	gi|212491|gb|AAA48996.1| ORF 138	2E-31	G

Contig	BLAST result	e value	Class
contig11045	gi|22164298|gb|AAM93654.1|AF483732_1 putative secreted NADH-ubiq... 138	2E-31	E
contig11385	gi|91090684|ref|XP_974597.1| PREDICTED: similar to AGAP005222-PB... 140	2E-31	F
contig06171	gi|196006812|ref|XP_002113272.1| hypothetical protein TRIADDRAFT... 120	1E-31	I
contig08433	gi|72088438|ref|XP_792502.1| PREDICTED: similar to high density ... 138	1E-31	I
contig06968	gi|193678979|ref|XP_001951009.1| PREDICTED: similar to predicted... 139	9E-32	G
contig02304	gi|110757125|ref|XP_625036.2| PREDICTED: similar to Phosphoribos... 140	4E-32	G
contig07207	gi|158289559|ref|XP_311260.4| AGAP000721-PA [*Anopheles gambiae* s... 141	2E-32	P
contig00526	gi|56756378|gb|AAW26362.1| SJCHGC08585 protein [*Schistosoma japo*... 142	1E-32	I
contig06194	gi|78190715|gb|ABB29679.1| methylthioadenosine phosphorylase MTA... 105	9E-33	G
contig11889	gi|114153138|gb|ABI52735.1| mitochondrial ATP synthase F chain [... 143	6E-33	PQ
contig02588	gi|195156388|ref|XP_002019082.1| GL25621 [*Drosophila persimilis*]... 143	4E-33	G
contig04675	gi|157127160|ref|XP_001661062.1| cysteine desulfurylase [*Aedes a*... 144	2E-33	G
contig08304	gi|189241870|ref|XP_971717.2| PREDICTED: similar to saccharopine... 93	2E-33	E
contig02843	gi|66508366|ref|XP_623071.1| PREDICTED: similar to Inosine-5-mon... 145	8E-34	G
contig00201	gi|149695380|ref|XP_001489937.1| PREDICTED: similar to Elastase-... 93	5E-34	H
contig11578	gi|198150845|gb|EAL29946.2| GA21583 [Drosophila pseudoobscura ps... 149	5E-34	P
contig10159	gi|110757127|ref|XP_001121693.1| PREDICTED: similar to CG5731-PA... 146	4E-34	E
contig04593	gi|156543929|ref|XP_001607212.1| PREDICTED: similar to ENSANGP00... 149	5E-35	I
contig06036	gi|91091714|ref|XP_966527.1| PREDICTED: similar to elongation of... 151	5E-35	C
contig07300	gi|91087539|ref|XP_970257.1| PREDICTED: similar to electron-tran... 150	4E-35	G
contig00369	gi|157167424|ref|XP_001660686.1| NADH:ubiquinone dehydrogenase, ... 151	3E-35	H
contig00702	gi|194749219|ref|XP_001957037.1| GF10225 [*Drosophila ananassae*] ... 91	3E-35	I
contig06578	gi|195026936|ref|XP_001986371.1| GH21324 [*Drosophila grimshawi*] ... 152	6E-36	I
contig00212	gi|193594270|ref|XP_001942800.1| PREDICTED: similar to S-adenosy... 154	2E-36	I
contig11959	gi|51011610|gb|AAT92214.1| cytochrome c oxidase subunit Va [Ixod... 157	8E-37	I
contig00375	gi|115665420|ref|XP_786125.2| PREDICTED: similar to putative gly... 107	6E-37	E
contig02327	gi|82878755|gb|AAW78557.2| vitellogenin [Dermacentor variabilis] 158	5E-37	I
contig00690	gi|56684769|gb|AAW22505.1| ferritin heavy chain-1b [*Carcinoscorp*... 160	1E-37	C
contig13356	gi|30692450|gb|AAP33409.1| cytochrome c oxidase subunit I [*Myrmi*... 162	1E-38	G
contig00072	gi|109463918|ref|XP_001056911.1| PREDICTED: similar to stearoyl-... 136	7E-39	C
contig01152	gi|194224071|ref|XP_001490939.2| PREDICTED: succinate dehydrogen... 162	6E-39	G
contig02729	gi|67083875|gb|AAY66872.1| ATP synthase D chain [*Ixodes scapularis*] 165	6E-39	PQ
contig01769	gi|110755373|ref|XP_396540.3| PREDICTED: similar to spinster CG8... 140	4E-39	RP
contig03462	gi|77955974|gb|ABB05539.1| mitochondrial manganese superoxide di... 165	2E-39	Q
contig05269	gi|161088766|gb|ABX57442.1| cytosolic malate dehydrogenase [Apor... 165	9E-40	I
contig11496	gi|170064241|ref|XP_001867443.1| 2-oxoglutarate dehydrogenase E1... 167	3E-40	E
contig11567	gi|157123148|ref|XP_001660031.1| hexokinase [*Aedes aegypti*] >gnl... 167	2E-40	E
contig02986	gi|66511507|ref|XP_623995.1| PREDICTED: similar to CG9307-PA iso... 168	1E-40	E
contig11865	gi|157073915|ref|NP_001096672.1| elastase 1, pancreatic [*Gallus* ... 148	1E-40	E
contig03924	gi|156538693|ref|XP_001607780.1| PREDICTED: similar to spermidin... 170	5E-41	IR
contig06418	gi|189524083|ref|XP_001918523.1| PREDICTED: hypothetical protein... 169	5E-41	G
contig04107	gi|91082287|ref|XP_973816.1| PREDICTED: similar to sodium/solute... 122	4E-41	H
contig02479	gi|194217822|ref|XP_001497298.2| PREDICTED: similar to Excitator... 108	1E-41	E
contig05025	gi|91083531|ref|XP_973193.1| PREDICTED: similar to predicted pro... 172	1E-41	G
contig00519	gi|168824098|gb|ACA30403.1| hexokinase [*Crassostrea gigas*] 172	7E-42	G
contig11199	gi|126338475|ref|XP_001364598.1| PREDICTED: similar to high dens... 173	6E-42	E
contig01171	gi|162462458|ref|NP_001105881.1| UMP synthase [*Zea mays*] >gnl|BL... 175	1E-42	E
contig01735	gi|2829707|sp|P81023|HBA_MELGA Hemoglobin subunit alpha-A (Hemog... 175	9E-43	I
contig03443	gi|47228564|emb|CAG05384.1| unnamed protein product [*Tetraodon n*... 175	9E-43	I
contig11637	gi|49257190|gb|AAH72658.1| Enoyl Coenzyme A hydratase, short cha... 137	4E-43	C
contig02519	gi|195349810|ref|XP_002041435.1| GM10152 [*Drosophila sechellia*] ... 178	2E-43	I

Contig	BLAST result	e value	Class
contig05359	gi|163916462|gb|AAI57294.1| Hydroxyacyl-Coenzyme A dehydrogenase... 180	6E-44	G
contig07915	gi|119590785|gb|EAW70379.1| NADH dehydrogenase (ubiquinone) Fe-S... 152	3E-45	C
contig12306	gi|39653771|ref|NP_945222.1| NADH dehydrogenase subunit 5 [*Haema*... 125	3E-45	I
contig00816	gi|91088235|ref|XP_973797.1| PREDICTED: similar to NADH-ubiquino... 150	9E-46	P
contig12639	gi|124076983|sp|P00040|CYC_SCHGR Cytochrome c 190	4E-47	C
contig13193	gi|27311165|ref|NP_758885.1| NADH dehydrogenase subunit 1 [*Varro*... 194	6E-48	QR
contig11544	gi|91092948|ref|XP_972400.1| PREDICTED: similar to Rieske iron-s... 201	5E-50	I
contig11525	gi|156397107|ref|XP_001637733.1| predicted protein [*Nematostella*... 160	3E-50	C
contig01007	gi|291866|gb|AAA35578.1| ATPase 201	2E-50	H
contig01937	gi|126338475|ref|XP_001364598.1| PREDICTED: similar to high dens... 203	2E-50	I
contig03555	gi|156537219|ref|XP_001605059.1| PREDICTED: similar to fatty aci... 205	1E-51	P
contig00381	gi|145579797|pdb|2JDI|G Chain G, Ground State Structure Of F1-At... 209	4E-52	HR
contig03238	gi|54887356|gb|AAH37009.1| Hydroxyacyl-Coenzyme A dehydrogenase/... 208	3E-52	G
contig00171	gi|194319795|gb|ABW82681.2| vitellogenin-2 precursor [*Dermacento*... 212	1E-52	C
contig11060	gi|66557932|ref|XP_623386.1| PREDICTED: similar to Glycogen phos... 208	1E-52	I
contig12032	gi|82878755|gb|AAW78557.2| vitellogenin [*Dermacentor variabilis*] 209	9E-53	P
contig04137	gi|187608195|ref|NP_001120566.1| hypothetical protein LOC1001457... 209	7E-53	E
contig00385	gi|195969366|gb|ACG60512.1| chitinase 1 precursor [*Scylla serrata*] 209	6E-53	F
contig05283	gi|158301525|ref|XP_321196.4| AGAP001870-PA [*Anopheles gambiae* s... 211	6E-53	H
contig04205	gi|157690439|gb|ABV65906.1| sodium/potassium-transporting ATPase... 154	3E-53	F
contig13158	gi|5835683|ref|NP_008499.1|COX2_15044 cytochrome c oxidase subun... 215	2E-54	E
contig05503	gi|109461932|ref|XP_001079287.1| PREDICTED: similar to glycogen ... 216	1E-54	P
contig08309	gi|151427590|tpd|FAA00352.1| TPA: predicted dual oxidase [*Apis m*... 217	3E-55	I
contig01709	gi|89271291|emb|CAJ82653.1| dihydropyrimidine dehydrogenase [*Xen*... 181	1E-55	C
contig11811	gi|195156303|ref|XP_002019040.1| GL26145 [*Drosophila persimilis*]... 220	6E-56	Q
contig08786	gi|156548615|ref|XP_001608103.1| PREDICTED: similar to CuZn supe... 222	3E-56	P
contig02897	gi|121543963|gb|ABM55646.1| putative vacuolar ATP synthase prote... 223	1E-56	I
contig11896	gi|91077486|ref|XP_968733.1| PREDICTED: similar to H+ transporti... 224	9E-57	F
contig00363	gi|2499486|sp|Q27543|K6PF_CALFI 6-phosphofructokinase (Phosphofr... 222	8E-57	C
contig01111	gi|170038460|ref|XP_001847068.1| deoxyuridine 5'-triphosphate nu... 225	4E-57	G
contig01510	gi|170172430|dbj|BAG12983.1| kynurenine aminotransferase [*Haemap*... 225	2E-57	C
contig01743	gi|91084123|ref|XP_967224.1| PREDICTED: similar to aspartate ami... 206	2E-57	G
contig12228	gi|189095643|ref|YP_001936484.1| cytochrome c oxidase subunit I ... 228	4E-58	P
contig02614	gi|47219329|emb|CAG10958.1| unnamed protein product [*Tetraodon n*... 227	3E-58	G
contig08892	gi|1213460|gb|AAB51350.1| C subunit of V-ATPase 229	7E-59	Q
contig01951	gi|67083945|gb|AAY66907.1| putative ATP synthase-like protein [*I*... 232	6E-59	C
contig03556	gi|158298125|ref|XP_318298.4| AGAP001065-PA [*Anopheles gambiae* s... 231	1E-59	I
contig00660	gi|126313584|ref|XP_001363206.1| PREDICTED: similar to flavin-co... 236	8E-61	E
contig00022	gi|161669226|gb|ABX75465.1| nucleoside diphosphate kinase [*Lycos*... 237	4E-61	E
contig00774	gi|193620315|ref|XP_001945213.1| PREDICTED: similar to AGAP00263... 146	4E-61	F
contig06731	gi|156537608|ref|XP_001607735.1| PREDICTED: similar to ENSANGP00... 236	3E-61	C
contig03297	gi|170042855|ref|XP_001849126.1| vacuolar ATP synthase subunit e... 241	2E-61	G
contig12646	gi|38707983|ref|NP_944590.1| aconitase 2, mitochondrial [*Danio r*... 238	1E-61	C
contig00610	gi|115898441|ref|XP_786111.2| PREDICTED: similar to fumarase [*St*... 239	5E-62	C
contig01532	gi|189525926|ref|XP_694336.3| PREDICTED: wu:fb81h03 [*Danio rerio*] 239	5E-62	E
contig01841	gi|34484219|gb|AAQ72744.1| sodium/potassium ATPase alpha subunit... 241	2E-62	Q
contig01974	gi|66535629|ref|XP_392950.2| PREDICTED: similar to CG3376-PA, is... 243	9E-63	I
contig04038	gi|50512854|gb|AAS93901.1| glycogen phosphorylase [*Crassostrea g*... 133	7E-63	G
contig01446	gi|45479211|gb|AAS66655.1| ferritin [*Hyalomma asiaticum asiaticum*] 247	4E-64	I
contig04670	gi|66523390|ref|XP_392776.2| PREDICTED: similar to Glutamate deh... 247	2E-64	G
contig08010	gi|82658272|ref|NP_001032494.1| hypothetical protein LOC641476 [... 247	2E-64	I

Contig	BLAST result	e value	Class
contig00394	gi|189233821|ref|XP_971721.2| PREDICTED: similar to conserved hy... 256	2E-65	C
contig12831	gi|91088621|ref|XP_967480.1| PREDICTED: similar to voltage-depen... 254	2E-65	CE
contig05135	gi|183448416|gb|ACC62887.1| glucose 6-phosphate dehydrogenase is... 251	1E-65	I
contig11892	gi|91077504|ref|XP_966852.1| PREDICTED: similar to saposin isofo... 230	5E-68	G
contig11295	gi|84095074|dbj|BAE66652.1| phenylalanine hydroxylase [*Papilio x*... 260	3E-68	G
contig06749	gi|156402676|ref|XP_001639716.1| predicted protein [*Nematostella*... 268	4E-70	P
contig06936	gi|195385958|ref|XP_002051671.1| GJ11069 [*Drosophila virilis*] >g... 266	3E-70	G
contig12538	gi|72053243|ref|XP_783757.1| PREDICTED: similar to GMPS protein ... 273	2E-71	C
contig04500	gi|198149572|gb|EDY73335.1| GA29299 [*Drosophila pseudoobscura* ps... 274	2E-72	C
contig13138	gi|66547465|ref|XP_392938.2| PREDICTED: similar to Glucose trans... 281	1E-73	IR
contig12246	gi|194226870|ref|XP_001917242.1| PREDICTED: similar to adenylosu... 263	5E-75	C
contig04596	gi|186920389|gb|ACC95446.1| glutamate dehydrogenase [*Litopenaeus*... 154	2E-75	G
contig01139	gi|46849491|dbj|BAD17955.1| glycinamide ribonucleotide synthetas... 291	1E-76	G
contig12226	gi|38564419|ref|NP_942572.1| choline transporter-like protein 2 ... 179	2E-77	G
contig11813	gi|156547189|ref|XP_001604156.1| PREDICTED: similar to sodium/po... 300	2E-79	I
contig02330	gi|194754279|ref|XP_001959423.1| GF12060 [*Drosophila ananassae*] ... 306	1E-81	CIQ
contig01232	gi|156552794|ref|XP_001600967.1| PREDICTED: similar to GA17603-P... 307	4E-82	P
contig01617	gi|156550600|ref|XP_001604166.1| PREDICTED: similar to inorganic... 281	4E-82	C
contig00570	gi|71895387|ref|NP_001025772.1| inosine monophosphate dehydrogen... 310	2E-82	E
contig02283	gi|189525094|ref|XP_001923821.1| PREDICTED: succinate-CoA ligase... 312	2E-83	E
contig01006	gi|189235816|ref|XP_972044.2| PREDICTED: similar to AGAP006936-P... 315	4E-84	C
contig02069	gi|126330256|ref|XP_001366956.1| PREDICTED: hypothetical protein... 249	4E-84	C
contig12613	gi|27311164|ref|NP_758884.1| cytochrome b [*Varroa destructor*] >g... 319	3E-85	I
contig12059	gi|2570843|gb|AAB82290.1| sarco/endoplasmic reticulum Ca2+-ATPas... 322	7E-86	E
contig11085	gi|167536447|ref|XP_001749895.1| predicted protein [*Monosiga bre*... 324	3E-87	C
contig12733	gi|118404104|ref|NP_001072376.1| 4-hydroxyphenylpyruvate dioxyge... 194	3E-88	C
contig01343	gi|5230695|gb|AAD40966.1|AF081807_1 cytochrome P450 4W1 [*Boophil*... 332	5E-89	G
contig02388	gi|157111333|ref|XP_001651490.1| cytosolic purine 5-nucleotidase... 332	3E-89	F
contig03685	gi|193706886|ref|XP_001950623.1| PREDICTED: similar to GA10597-P... 333	1E-89	H
contig02154	gi|114319091|gb|ABI63547.1| triosephosphate isomerase [*Blattella*... 337	1E-90	P
contig01207	gi|91086519|ref|XP_971755.1| PREDICTED: similar to vesicle amine... 337	9E-91	C
contig01461	gi|170032167|ref|XP_001843954.1| conserved hypothetical protein ... 231	3E-91	P
contig01818	gi|91078838|ref|XP_971511.1| PREDICTED: similar to AGAP007285-PA... 338	1E-91	H
contig00502	gi|73979739|ref|XP_856678.1| PREDICTED: similar to Ornithine dec... 345	8E-93	E
contig00484	gi|115495787|ref|NP_001070100.1| solute carrier family 25 (mitoc... 355	9E-96	I
contig05520	gi|110769804|ref|XP_624006.2| PREDICTED: similar to dre4 CG1828-... 338	6E-96	Q
contig03159	gi|197632181|gb|ACH70814.1| S-adenosylhomocysteine hydrolase [*Sa*... 356	1E-96	I
contig04951	gi|114052783|ref|NP_001040279.1| serine hydroxymethyltransferase... 361	8E-98	C
contig00435	gi|66509679|ref|XP_395764.2| PREDICTED: similar to CTP:phosphoch... 369	1E-100	IG
contig00890	gi|437279|gb|AAB86499.1| stearoyl-CoA desaturase [*Amblyomma amer*... 369	1E-100	C
contig02767	gi|148223856|ref|NP_001087698.1| MGC83352 protein [*Xenopus laevi*... 368	1E-100	I
contig01715	gi|194765671|ref|XP_001964950.1| GF22842 [*Drosophila ananassae*] ... 379	1E-103	C
contig01584	gi|189239301|ref|XP_971644.2| PREDICTED: similar to utp-glucose-... 392	1E-107	I
contig04889	gi|114052230|ref|NP_001040132.1| hydroxyacyl-coenzyme A dehydrog... 354	1E-108	F
contig11916	gi|72053243|ref|XP_783757.1| PREDICTED: similar to GMPS protein ... 394	1E-108	C
contig00877	gi|41054619|ref|NP_955871.1| phosphoglycerate dehydrogenase [*Dan*... 397	1E-109	C
contig01427	gi|25090938|sp|Q27128|PAPSS_URECA Bifunctional 3'-phosphoadenosi... 252	1E-114	C
contig12001	gi|50758110|ref|XP_415765.1| PREDICTED: similar to Malate dehydr... 326	1E-114	I
contig13207	gi|194241600|gb|ACF35055.1| hemelipoglycoprotein precursor 2 [*De*... 159	1E-116	I
contig00376	gi|21450129|ref|NP_659033.1| acetyl-Coenzyme A acetyltransferase... 266	1E-120	F
contig00872	gi|118101892|ref|XP_417879.2| PREDICTED: similar to MGC64378 pro... 419	1E-123	G

Contig	BLAST result	e value	Class
contig02591	gi|157106042|ref|XP_001649141.1| transketolase [*Aedes aegypti*] >... 454	1E-126	P
contig00067	gi|158631166|ref|NP_001037072.1| ADP/ATP translocase [*Bombyx mor*... 391	1E-127	G
contig11557	gi|74950265|sp|Q4U3L0|G3P_GLOMM Glyceraldehyde-3-phosphate dehyd... 406	1E-128	I
contig11619	gi|72010168|ref|XP_783048.1| PREDICTED: similar to Slc25a3-prov ... 464	1E-128	QI
contig01072	gi|158292977|ref|XP_314288.4| AGAP004890-PB [*Anopheles gambiae* s... 480	1E-133	I
contig13306	gi|195114014|ref|XP_002001562.1| GI16461 [*Drosophila mojavensis*]... 275	1E-133	C
contig00578	gi|92090585|sp|Q27238|ADT1_ANOGA ADP,ATP carrier protein 1 (ADP/... 501	1E-140	F
contig00885	gi|25453072|sp|Q95V58|KARG_ARTSF Arginine kinase (AK) >gnl|BL_OR... 516	1E-144	C
contig11570	gi|195430072|ref|XP_002063081.1| GK21733 [*Drosophila willistoni*]... 287	1E-145	P
contig00631	gi|115948338|ref|XP_001175657.1| PREDICTED: similar to 5-aminoim... 521	1E-146	IR
contig11550	gi|110748949|ref|XP_623342.2| PREDICTED: similar to Aldolase CG6... 532	1E-149	C
contig01932	gi|58390364|ref|XP_317672.2| AGAP007827-PA [*Anopheles gambiae* st... 548	1E-154	P
contig11287	gi|157133341|ref|XP_001656210.1| citrate synthase [*Aedes aegypti*... 574	1E-161	F
contig01083	gi|91092172|ref|XP_968236.1| PREDICTED: similar to Type III alco... 352	1E-166	C
contig01267	gi|148237376|ref|NP_001086928.1| phosphoglycerate kinase 2 [*Xeno*... 588	1E-166	F
contig02408	gi|157111261|ref|XP_001651458.1| ATP synthase subunit beta vacuo... 399	1E-167	P
contig01077	gi|158290905|ref|XP_312441.4| AGAP002499-PA [*Anopheles gambiae* s... 595	1E-168	F
contig11592	gi|169260101|gb|ACA51932.1| arginine kinase [*Metapenaeus ensis*] 396	1E-170	I
contig12598	gi|157690439|gb|ABV65906.1| sodium/potassium-transporting ATPase... 612	1E-173	P
contig00859	gi|170064276|ref|XP_001867458.1| glutamate carboxypeptidase [*Cul*... 615	1E-174	E
contig01816	gi|157137937|ref|XP_001664086.1| aconitase, mitochondrial [*Aedes*... 464	1E-178	C
contig11897	gi|194319795|gb|ABW82681.2| vitellogenin-2 precursor [*Dermacento*... 636	1E-180	C
contig00165	gi|149413032|ref|XP_001506958.1| PREDICTED: similar to ATP synth... 863	0	CE
contig00870	gi|158866359|gb|ABW82155.1| catalase [*Fenneropenaeus chinensis*] 681	0	C
contig02962	gi|157133637|ref|XP_001662952.1| bifunctional purine biosynthesi... 276	0	G
contig03582	gi|160898730|ref|YP_001564312.1| hypothetical protein Daci_3289 ... 21	0	G
contig09482	gi|196011112|ref|XP_002115420.1| hypothetical protein TRIADDRAFT... 40	0	E
contig12013	gi|82878755|gb|AAW78557.2| vitellogenin [*Dermacentor variabilis*] 831	0	G
contig13231	gi|134143225|gb|ABO61883.1| phosphoenolpyruvate carboxykinase [R... 884	0	P
contig13317	gi|194319795|gb|ABW82681.2| vitellogenin-2 precursor [*Dermacento*... 759	0	H
contig13348	gi|147905995|ref|NP_001083034.1| hypothetical protein LOC1000387... 781	0	P
contig00751	No hits found		IR
contig01516	No hits found		E
contig01547	No hits found		I
contig01950	No hits found		C
contig03479	No hits found		IR
contig04021	No hits found		E
contig04371	No hits found		C
contig04512	No hits found		Q
contig06567	No hits found		Q
contig06596	No hits found		G
contig08015	No hits found		C
contig08406	No hits found		C
contig09560	No hits found		I
contig09833	No hits found		I
contig09912	No hits found		C
contig10647	No hits found		I
contig10694	No hits found		H
contig10832	No hits found		I
contig11583	No hits found		C
contig12427	No hits found		E

Contig	BLAST result	e value	Class
contig12439	No hits found		I
contig12811	No hits found		C
contig12857	No hits found		C
contig12975	No hits found		C

Appendix 3:

Illumina Solexa supplementary data.

Table showing all the contigs that had count data in each sample and had a significant fold change of more than three.

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig12543	2.6E-04	110.853	gi|17570195|ref|NP_508868.1| VITellogenin structural genes (yolk... 60	28738	99606	52525	561	361	545
contig12032	0.00511	69.317	gi|82878755|gb|AAW78557.2| vitellogenin [*Dermacentor variabilis*] 209	7916	2465	2294	150	16	56
contig12020	0.00587	58.038	No hits found	11528	3657	8541	473	66	59
contig12579	6.5E-05	55.864	gi|169215918|ref|XP_001718712.1| PREDICTED: hypothetical protein... 42	11859	7087	10710	191	124	218
contig11532	0.02143	51.208	gi|125524395|gb|EAY72509.1| hypothetical protein OsI_000356 [Ory... 33	8313	36848	39333	2757	115	283
contig00712	0.00012	39.675	gi|167035216|ref|YP_001670447.1| hypothetical protein PputGB1_42... 35	9327	11563	15466	225	271	438
contig00149	1.7E-05	38.910	No hits found	6404	7744	8025	224	145	208
contig13089	0.03095	37.151	gi|119628581|gb|EAX08176.1| hCG2038677 [*Homo sapiens*] 38	23667	1231	2065	332	38	93
contig12724	0.00204	36.924	gi|74267388|dbj|BAE44191.1| cuticular protein [*Tachypleus triden*... 50	1071	1231	2291	25	25	96
contig01395	8.2E-04	35.517	gi|119715610|ref|YP_922575.1| hypothetical protein Noca_1374 [*No*... 35	1471	1353	1569	24	88	33
contig12891	0.02325	29.318	gi|153829295|ref|ZP_01981962.1| MshQ [*Vibrio cholerae* 623-39] >g... 36	14901	51336	50954	6080	365	697
contig13001	0.02963	27.656	No hits found	734	1562	1750	304	24	13
contig12013	0.00267	26.812	gi|82878755|gb|AAW78557.2| vitellogenin [*Dermacentor variabilis*] 831	127950	71499	50643	5890	1400	2915
contig00171	0.01360	24.596	gi|194319795|gb|ABW82681.2| vitellogenin-2 precursor [*Dermacento*... 212	101586	20153	17416	3380	583	1216
contig12861	0.00126	21.875	gi|158316180|ref|YP_001508688.1| hypothetical protein Franean1_4... 35	648	1276	1940	43	44	81
contig12148	1.5E-04	21.540	gi|170696288|ref|ZP_02887419.1| transcriptional regulator, winge... 34	2905	1962	3000	85	136	148
contig01907	0.01108	19.885	gi|114153294|gb|ABI52813.1| GGY domain protein [*Argas monolakensis*] 39	109	540	778	13	32	14
contig07410	0.01253	19.579	gi|212491|gb|AAA48996.1| ORF 83	561	735	3128	133	38	34
contig04772	0.00521	17.297	No hits found	1530	1486	4942	85	103	248
contig01984	0.01228	16.398	No hits found	1460	1124	2336	50	322	54
contig12559	0.04277	15.478	gi|195567675|ref|XP_002107384.1| GD17434 [*Drosophila simulans*] >... 44	607	830	1281	344	22	23
contig04119	0.01044	13.771	No hits found	348	1370	948	104	64	26
contig00317	0.00433	13.486	No hits found	369	532	1417	45	36	70
contig00690	9.2E-04	12.945	gi|56684769|gb|AAW22505.1| ferritin heavy chain-1b [*Carcinoscorp*... 160	462	270	378	19	44	26
contig05591	0.02280	12.219	No hits found	556	612	605	14	155	52
contig13306	9.8E-04	11.777	gi|195114014|ref|XP_002001562.1| GI16461 [*Drosophila mojavensis*]... 275	2917	2853	4239	341	168	377

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig02256	0.02389	11.114	gi|189202442|ref|XP_001937557.1| conserved hypothetical protein ... 38	869	297	782	35	168	25
contig06158	0.04003	10.235	No hits found	302	870	474	15	44	176
contig12606	0.00500	10.089	gi|109506318|ref|XP_226158.4| PREDICTED: similar to Mucin-2 prec... 57	2532	753	2552	158	153	196
contig12810	0.00184	9.969	gi|114675653|ref|XP_001170388.1| PREDICTED: deoxyribonuclease II... 119	1910	833	1827	172	104	164
contig00670	0.00127	9.900	gi|72008176|ref|XP_780713.1| PREDICTED: similar to cathepsin L [... 377	3956	4789	8377	741	541	408
contig12371	0.03655	9.828	gi|149723703|ref|XP_001489791.1| PREDICTED: granulin [*Equus caba*... 45	468	59	363	16	15	44
contig13131	0.00447	9.699	gi|91081217|ref|XP_975622.1| PREDICTED: similar to ecdysteroid r... 116	823	1202	2413	89	200	147
contig13200	0.03687	9.619	gi|111220888|ref|YP_711682.1| 1-hydroxy-2-methyl-2-(E)-butenyl 4... 43	3675	6429	36634	1589	878	697
contig13074	0.01140	9.557	gi|47230018|emb|CAG10432.1| unnamed protein product [*Tetraodon n*... 169	951	534	1164	50	215	63
contig11938	0.04833	8.563	gi|161788938|dbj|BAF95090.1| 2nd tick legumain [*Haemaphysalis lo*... 77	586	140	225	99	11	27
contig11417	4.1E-04	8.536	gi|91080885|ref|XP_972748.1| PREDICTED: similar to GA14321-PA [*T*... 37	97	73	137	12	10	13
contig11374	0.04441	8.515	No hits found	689	126	98	13	53	20
contig11787	3.5E-04	8.513	gi|15219757|ref|NP_176856.1| unknown protein [*Arabidopsis thalia*... 42	74679	140492	100406	12198	12937	10821
contig00726	0.03861	8.411	gi|45386073|gb|AAS59854.1| NADH-ubiquinone oxidoreductase [*Ornit*... 53	681	233	408	39	155	18
contig05927	0.02458	8.041	No hits found	639	102	131	23	34	21
contig11836	0.00187	7.939	gi|40548304|ref|NP_954972.1| carboxypeptidase, vitellogenic-like... 303	4773	1846	2783	321	376	406
contig12768	6.5E-04	7.628	gi|196236958|ref|ZP_03135749.1| conserved hypothetical protein [... 49	4136	3918	7235	698	550	688
contig05241	0.02602	7.582	gi|169170573|ref|XP_001716045.1| PREDICTED: hypothetical protein... 34	727	308	147	31	28	87
contig12017	0.00561	7.522	No hits found	2824	1319	2093	350	145	361
contig12038	0.03652	7.274	gi|1236646|gb|AAC48775.1| ch4 and secrete domains of swine IgM [... 34	491	126	75	41	21	14
contig08739	0.03324	7.252	gi|186686156|ref|YP_001869352.1| hypothetical protein Npun_F6121... 33	2117	401	483	138	147	53
contig13173	6.3E-05	7.215	gi|1389604|gb|AAC59905.1| acetylcholinesterase 186	13321	12603	16570	2172	2027	1682
contig02590	0.04679	7.086	gi|51949802|gb|AAU14833.1| adenosine kinase isoform 2S [*Nicotian*... 132	201	380	598	15	107	80
contig01314	0.03827	7.082	gi|157108382|ref|XP_001650201.1| seryl-tRNA synthetase [*Aedes ae*... 105	532	133	64	25	30	17
contig12549	0.04210	7.079	No hits found	1237	254	271	127	63	30
contig01853	0.01540	6.994	gi|66508914|ref|XP_623207.1| PREDICTED: similar to CG10527-PA is... 100	948	234	671	49	107	83
contig12598	0.03671	6.850	gi|157690439|gb|ABV65906.1| sodium/potassium-transporting ATPase... 612	298	36	110	18	12	17

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig00304	0.00764	6.641	gi|153832046|ref|ZP_01984713.1| pyruvate/2-oxoglutarate dehydrog... 43	371	189	275	55	21	57
contig00102	0.04250	6.509	gi|187598503|ref|ZP_02984755.1| hypothetical protein GdiaDRAFT_3... 52	557	593	206	74	145	23
contig12507	0.03377	6.448	gi|81096421|ref|ZP_00874766.1| hypothetical protein SsuiDRAFT_16... 49	3037	853	456	122	230	157
contig05426	0.00816	6.422	gi|157676771|emb|CAP08020.1| unnamed protein product [*Danio rerio*] 36	678	420	730	150	48	109
contig11590	5.7E-04	6.225	gi|149287022|gb|ABR23410.1| p8 nuclear protein [*Ornithodoros par*... 80	7885	6337	6074	776	1263	1284
contig00207	0.02356	6.088	gi|46111825|ref|XP_382970.1| hypothetical protein FG02794.1 [*Gib*... 38	148	180	247	81	20	18
contig04973	0.01136	6.008	gi|14485281|ref|NP_058968.14| aldehyde dehydrogenase family 1, s... 95	135	66	184	30	21	12
contig00873	0.01953	5.856	gi|2665388|dbj|BAA23764.1| cyclophilin G [*Tachypleus tridentatus*] 251	446	148	511	34	81	61
contig13129	0.00650	5.688	gi|118429511|gb|ABK91803.1| aspartic protease precursor [*Clonorc*... 177	1625	666	1574	226	266	154
contig00052	0.01387	5.632	gi|111022465|ref|YP_705437.1| chaperone protein [*Rhodococcus sp*.... 35	135	246	377	32	30	73
contig02996	0.01838	5.549	gi|195376021|ref|XP_002046795.1| GJ12289 [*Drosophila virilis*] >g... 39	148	167	132	62	22	14
contig01412	0.01832	5.453	No hits found	807	776	858	62	244	219
contig11508	0.04393	5.440	gi|157748651|ref|XP_001669420.1| Hypothetical protein CBG19758 [... 35	2352	355	797	122	143	237
contig01148	0.04266	5.427	No hits found	588	261	309	27	67	164
contig11293	0.00662	5.372	gi|148717315|dbj|BAF63671.1| protein disulfide isomerase-2 [*Haem*... 385	1079	991	1433	118	261	321
contig07771	0.01428	5.339	No hits found	179	110	113	42	29	12
contig01438	0.03987	5.321	gi|150864759|ref|XP_001383727.2| hypothetical protein PICST_4371... 35	1259	347	305	53	162	103
contig12246	0.00141	5.285	gi|194226870|ref|XP_001917242.1| PREDICTED: similar to adenylosu... 263	800	656	796	102	136	204
contig00176	0.03015	5.179	gi|67083759|gb|AAY66814.1| selenium dependent salivary glutathio... 188	2194	617	599	156	126	297
contig02485	0.01107	5.175	gi|66524917|ref|XP_393112.2| PREDICTED: similar to 26S proteasom... 219	475	260	152	37	60	61
contig02881	0.04351	5.157	gi|67920250|ref|ZP_00513770.1| conserved hypothetical protein [*C*... 33	4562	929	1127	296	530	222
contig12224	0.03416	5.132	gi|115655582|ref|XP_780679.2| PREDICTED: similar to granulin [*St*... 106	2004	375	843	202	200	116
contig07518	0.02419	5.075	gi|196001653|ref|XP_002110694.1| hypothetical protein TRIADDRAFT... 45	491	139	121	32	47	42
contig12061	0.03293	5.044	gi|8050849|gb|AAF71775.1|AF263912_14 NysB [*Streptomyces noursei*] 38	1419	368	1076	91	228	211
contig13159	0.02917	5.006	No hits found	87	274	78	15	38	26
contig13068	0.00303	4.948	gi|27733415|gb|AAO21505.1|AF413064_1 serpin 3a [*Manduca sexta*] >... 35	86	141	113	26	29	15
contig03858	0.03691	4.932	gi|149634388|ref|XP_001506411.1| PREDICTED: similar to alpha-asp... 93	278	517	579	35	133	149

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig12705	0.02520	4.922	gi|189208430|ref|XP_001940548.1| laccase-1 precursor [*Pyrenophor*... 26	7114	1846	2340	846	686	444
contig01538	0.00647	4.922	gi|47522760|ref|NP_999131.1| long-chain 3-ketoacyl-CoA thiolase ... 129	236	261	262	36	94	40
contig00466	0.01495	4.879	gi|74096057|ref|NP_001027676.1| sec61 protein [*Ciona intestinali*... 64	480	257	337	37	118	82
contig06291	0.02386	4.875	gi|171689310|ref|XP_001909595.1| unnamed protein product [*Podosp*... 34	123	173	172	14	37	61
contig12253	0.01082	4.862	gi|123959678|gb|AAI28967.1| LOC100037110 protein [*Xenopus laevis*] 65	595	237	301	47	81	97
contig02168	0.04722	4.862	gi|169218160|ref|XP_001719783.1| PREDICTED: similar to KLHL23 pr... 30	576	157	113	27	54	61
contig08063	0.03875	4.841	gi|196000721|ref|XP_002110228.1| expressed hypothetical protein ... 45	227	45	179	20	31	26
contig01772	0.00507	4.839	gi|169217278|ref|XP_001732938.1| PREDICTED: hypothetical protein... 34	1855	924	873	316	209	200
contig12894	0.00395	4.807	gi|114153234|gb|ABI52783.1| mucin peritrophin salivary protein [... 70	10902	25028	18318	3651	2948	4181
contig02157	7.2E-04	4.781	gi|67083785|gb|AAY66827.1| secreted salivary protein [*Ixodes sca*... 48	2033	2647	2771	406	517	650
contig02767	0.01405	4.778	gi|148223856|ref|NP_001087698.1| MGC83352 protein [*Xenopus laevi*... 368	395	317	609	134	111	47
contig00262	0.00972	4.754	gi|153791210|ref|NP_001014420.2| taste receptor protein 1 [*Mus m*... 36	11438	3970	9425	1483	1872	1435
contig03656	0.04663	4.731	gi|195108637|ref|XP_001998899.1| GI24219 [*Drosophila mojavensis*]... 43	423	417	774	55	80	293
contig11681	0.00141	4.723	No hits found	270	184	294	55	63	40
contig12243	0.02839	4.668	gi|39725977|gb|AAR29940.1| calreticulin [*Boophilus microplus*] 323	652	205	441	45	116	111
contig00022	0.01571	4.648	gi|161669226|gb|ABX75465.1| nucleoside diphosphate kinase [*Lycos*... 237	2017	723	1252	165	365	302
contig07116	0.00447	4.637	No hits found	318	384	429	54	128	76
contig00916	0.03627	4.637	gi|50251506|dbj|BAD28867.1| aminotransferase-like [*Oryza sativa* ... 36	240	123	107	15	32	66
contig03414	0.04484	4.554	gi|149898803|gb|ABR27863.1| Sec61 protein translocation complex ... 87	655	183	124	49	73	44
contig05486	0.04412	4.530	gi|194765045|ref|XP_001964638.1| GF23289 [*Drosophila ananassae*] ... 57	1200	249	713	194	85	139
contig02324	0.00413	4.524	gi|41617918|tpg|DAA02898.1| TPA: TPA_inf: HDC09484 [*Drosophila m*... 33	236	544	385	70	93	82
contig12045	0.04927	4.516	gi|149031098|gb|EDL86125.1| rCG37365 [*Rattus norvegicus*] 35	552	705	1264	101	456	116
contig11943	0.00374	4.511	gi|171912587|ref|ZP_02928057.1| hypothetical protein VspiD_15445... 33	752	516	833	102	194	178
contig03269	0.00197	4.501	No hits found	2036	3587	2760	784	530	532
contig02067	0.02818	4.486	gi|145340986|ref|XP_001415597.1| predicted protein [*Ostreococcus*... 33	1376	428	742	109	294	151
contig11625	0.03843	4.485	gi|170030742|ref|XP_001843247.1| proteasome subunit alpha type 6... 384	1431	288	500	100	136	168
contig10198	0.00846	4.439	gi|156546284|ref|XP_001605934.1| PREDICTED: similar to ENSANGP00... 34	306	235	354	68	40	107

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig00556	0.03373	4.415	gi|195396577|ref|XP_002056907.1| GJ16636 [*Drosophila virilis*] >g... 33	875	253	280	57	95	133
contig00188	0.02877	4.408	gi|196476807|gb|ACG76267.1| histone 2B [*Amblyomma americanum*] 182	267	307	451	109	120	33
contig00212	0.00561	4.364	gi|193594270|ref|XP_001942800.1| PREDICTED: similar to S-adenosy... 154	357	180	268	85	46	53
contig00498	0.00261	4.352	gi|88602953|ref|YP_503131.1| HNH endonuclease [*Methanospirillum* ... 36	4921	2929	3131	793	1072	644
contig06286	0.02872	4.290	gi|169640128|gb|ACA61137.1| cellulase [uncultured microorganism] 36	108	180	327	24	61	55
contig12692	0.01634	4.257	No hits found	181	103	139	20	28	60
contig12538	0.04258	4.165	gi|72053243|ref|XP_783757.1| PREDICTED: similar to GMPS protein ... 273	251	315	472	35	90	164
contig02748	0.01514	4.097	gi|194218564|ref|XP_001917008.1| PREDICTED: low density lipoprot... 87	694	365	905	97	179	192
contig01006	0.02642	4.051	gi|189235816|ref|XP_972044.2| PREDICTED: similar to AGAP006936-P... 315	1677	546	583	248	233	139
contig04737	0.00861	4.042	No hits found	63	62	102	13	16	29
contig07176	0.00381	3.996	gi|28422163|gb|AAH46839.1| Plk-prov protein [*Xenopus laevis*] 147	885	1163	1322	209	405	252
contig10262	0.00272	3.991	gi|74318688|ref|YP_316428.1| metal dependent phosphohydrolase, H... 36	176	117	129	25	44	38
contig00165	0.00930	3.978	gi|149413032|ref|XP_001506958.1| PREDICTED: similar to ATP synth... 863	6398	2898	3364	1381	886	810
contig13261	3.4E-04	3.972	gi|157776976|ref|XP_001666554.1| hypothetical protein CBG15153 [... 65	630	597	700	181	180	129
contig04318	0.01009	3.965	gi|194225289|ref|XP_001494645.2| PREDICTED: similar to Fibulin 5... 60	2454	2237	5055	950	825	568
contig13273	0.01583	3.954	No hits found	513	473	719	152	235	79
contig00156	0.01187	3.900	gi|118104159|ref|XP_425038.2| PREDICTED: similar to cathepsin L ... 96	2397	1044	2790	447	543	485
contig01743	0.03107	3.891	gi|91084123|ref|XP_967224.1| PREDICTED: similar to aspartate ami... 206	339	124	128	45	29	70
contig02329	0.04537	3.888	gi|72042689|ref|XP_794417.1| PREDICTED: similar to Retinol dehyd... 120	62	119	182	17	24	56
contig00859	0.01226	3.867	gi|170064276|ref|XP_001867458.1| glutamate carboxypeptidase [Cul... 615	364	191	377	115	73	54
contig13232	0.03540	3.848	gi|66506394|ref|XP_624700.1| PREDICTED: similar to CG31618-PA [A... 160	40471	14587	10732	5116	6103	3562
contig07208	0.00411	3.846	gi|118462588|ref|YP_882079.1| CDP-alcohol phosphatidyltransferas... 37	499	331	460	84	100	159
contig11796	0.00432	3.842	gi|82705844|ref|XP_727137.1| hypothetical protein PY06477 [*Plasm*... 39	1376	1865	1151	278	359	522
contig11910	0.01826	3.837	gi|156372710|ref|XP_001629179.1| predicted protein [*Nematostella*... 379	1023	1344	3089	366	520	395
contig06536	0.03600	3.803	gi|58387114|ref|XP_315337.2| AGAP005323-PB [*Anopheles gambiae* st... 75	126	32	55	16	12	21
contig13080	0.01102	3.773	gi|45269081|gb|AAS55945.1| actin [*Ornithodoros moubata*] 742	16230	7077	9123	1932	3114	3244
contig04680	0.00770	3.750	gi|124487744|gb|ABN11959.1| putative chaperonin subunit 6a zeta ... 82	443	351	446	125	157	67

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig13130	0.02822	3.743	gi|115931821|ref|XP_001180548.1| PREDICTED: hypothetical protein... 44	1502	450	756	225	283	153
contig04085	4.2E-04	3.688	gi|170094979|ref|XP_001878710.1| predicted protein [*Laccaria bic*... 33	421	449	326	97	123	103
contig12240	0.04692	3.685	gi|33348836|gb|AAQ16118.1| cathepsin L-like cysteine proteinase ... 123	1944	2014	3268	286	723	1237
contig03700	0.00426	3.684	gi|191171858|ref|ZP_03033404.1| Cps2D [*Escherichia coli* F11] >gn... 33	767	464	416	141	178	118
contig06739	0.02577	3.669	gi|169206246|ref|XP_001716022.1| PREDICTED: hypothetical protein... 48	191	145	78	60	27	27
contig13043	0.04933	3.654	No hits found	1925	652	815	170	514	240
contig09987	0.02739	3.598	gi|114153104|gb|ABI52718.1| metalloprotease [*Argas monolakensis*] 34	105	66	71	44	16	15
contig11620	0.00665	3.591	gi|149287114|gb|ABR23456.1| cytochrome c oxidase polyprotein IV ... 50	237	202	392	65	99	63
contig00224	0.01188	3.585	gi|39725965|gb|AAR29934.1| calreticulin [*Amblyomma cooperi*] 546	1562	1271	2518	725	396	378
contig04408	0.03623	3.580	gi|169213506|ref|XP_001719061.1| PREDICTED: hypothetical protein... 42	1699	1004	1237	173	422	630
contig12001	0.00722	3.529	gi|50758110|ref|XP_415765.1| PREDICTED: similar to Malate dehydr... 326	769	1149	1243	436	246	233
contig09563	0.00459	3.514	gi|169213190|ref|XP_001714612.1| PREDICTED: hypothetical protein... 34	74	48	61	24	13	16
contig00950	0.01114	3.508	gi|170062080|ref|XP_001866514.1| conserved hypothetical protein ... 33	3812	3698	2929	576	1310	1268
contig05646	0.02734	3.490	gi|194227065|ref|XP_001915913.1| PREDICTED: similar to Zinc fing... 33	119	112	252	62	49	26
contig13208	0.03647	3.476	No hits found	2862	7833	7447	1181	2518	1337
contig01639	0.03029	3.475	No hits found	155	162	360	64	37	91
contig04310	0.00134	3.443	gi|158287073|ref|XP_309120.3| AGAP000935-PA [*Anopheles gambiae* s... 305	409	371	600	127	122	144
contig07136	0.02224	3.442	gi|86355535|ref|YP_473203.1| P49 [*Hyphantria cunea nucleopolyhed*... 33	279	178	142	32	74	73
contig12842	0.01013	3.435	gi|148709239|gb|EDL41185.1| Max dimerization protein 3, isoform ... 29	1119	2370	2533	612	547	495
contig12071	0.00253	3.434	gi|109118856|ref|XP_001109939.1| PREDICTED: acid alpha-glucosida... 61	1354	1749	1891	370	628	476
contig11842	0.02746	3.419	gi|98986297|dbj|BAE94536.1| hypothetical protein [*Colletotrichum*... 35	94	79	135	16	49	32
contig04095	0.03214	3.409	No hits found	100	136	164	19	52	57
contig01211	0.02643	3.390	gi|125586705|gb|EAZ27369.1| hypothetical protein OsJ_010852 [*Ory*... 40	1261	655	928	491	212	189
contig00921	0.02573	3.389	gi|68171402|ref|ZP_00544794.1| Glyceraldehyde-3-phosphate dehydr... 35	565	228	261	103	131	64
contig12736	0.02998	3.388	gi|167534260|ref|XP_001748808.1| predicted protein [*Monosiga bre*... 72	1492	3054	4511	571	897	1032
contig12241	0.04574	3.364	gi|91084417|ref|XP_967827.1| PREDICTED: similar to chromobox-lik... 104	160	339	348	76	145	45
contig00877	0.04542	3.355	gi|41054619|ref|NP_955871.1| phosphoglycerate dehydrogenase [*Dan*... 397	582	745	1166	118	414	274

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig12206	0.01696	3.345	No hits found	763	364	304	146	150	103
contig11867	0.02125	3.323	No hits found	60	37	100	14	18	24
contig03781	0.04544	3.323	gi|94733117|emb|CAK04429.1| novel protein similar to vertebrate ... 50	239	188	238	31	74	127
contig10333	0.04019	3.316	gi|119577990|gb|EAW57586.1| chromosome 17 open reading frame 78,... 33	554	235	243	56	149	104
contig00470	7.9E-05	3.313	gi|156353921|ref|XP_001623156.1| predicted protein [*Nematostella*... 66	5270	5577	4710	1466	1497	1735
contig11441	0.00473	3.293	gi|148657070|ref|YP_001277275.1| putative endoribonuclease L-PSP... 143	1935	3275	3082	647	1005	841
contig00543	0.00725	3.287	gi|170596615|ref|XP_001902831.1| FKBP-type peptidyl-prolyl cis-t... 103	1640	1074	1284	276	418	552
contig00938	0.00913	3.282	gi|169175412|ref|ZP_02837264.1| putative serine protease, subtil... 35	1950	3101	3538	620	859	1136
contig10397	0.03888	3.277	gi|195444800|ref|XP_002070035.1| GK11242 [*Drosophila willistoni*]... 33	208	479	716	102	167	119
contig00223	9.1E-04	3.269	No hits found	3822	3836	5632	1437	1264	1301
contig03105	0.01566	3.265	gi|84570464|dbj|BAE72662.1| mucin [*Haemaphysalis longicornis*] 54	6450	4716	3554	964	1468	2194
contig11550	0.01678	3.262	gi|110748949|ref|XP_623342.2| PREDICTED: similar to Aldolase CG6... 532	631	244	406	139	135	96
contig05201	0.00871	3.260	No hits found	1050	2297	1241	412	503	417
contig13340	0.00959	3.259	gi|157757981|ref|XP_001671334.1| Hypothetical protein CBG01569 [... 37	904	1778	1528	367	557	347
contig11595	0.01944	3.258	gi|193608341|ref|XP_001948728.1| PREDICTED: similar to conserved... 45	6616	7146	12819	1641	3071	3478
contig11535	0.02715	3.253	gi|27311161|ref|NP_758881.1| NADH dehydrogenase subunit 4 [*Varro*... 139	316953	461095	444976	67028	202999	138882
contig01111	0.01937	3.235	gi|170038460|ref|XP_001847068.1| deoxyuridine 5'-triphosphate nu... 225	1299	1044	1227	200	477	515
contig01770	0.00166	3.234	gi|189240738|ref|XP_968166.2| PREDICTED: similar to AGAP007574-P... 107	586	461	712	209	152	179
contig13293	0.02813	3.229	gi|159470549|ref|XP_001693419.1| predicted protein [*Chlamydomona*... 39	65	92	136	21	50	23
contig00629	0.01278	3.216	gi|167536992|ref|XP_001750166.1| predicted protein [*Monosiga bre*... 30	2859	1319	1307	620	512	467
contig09309	0.04289	3.205	No hits found	427	201	348	168	100	54
contig03108	0.02505	3.200	gi|149287082|gb|ABR23440.1| RNA binding protein [*Ornithodoros pa*... 75	906	1067	1032	239	601	212
contig07552	0.03152	3.180	gi|168062615|ref|XP_001783274.1| predicted protein [*Physcomitrel*... 139	158	72	58	38	27	20
contig03260	0.04405	3.156	gi|195153445|ref|XP_002017636.1| GL17205 [*Drosophila persimilis*]... 153	128	78	120	59	17	38
contig10131	0.03695	3.150	gi|170064624|ref|XP_001867602.1| mitochondrial GTPase 1 [*Culex q*... 50	245	402	493	86	215	84
contig01484	0.02398	3.137	gi|157331182|emb|CAO63589.1| unnamed protein product [*Vitis vini*... 39	164	186	439	77	88	64

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig00345	0.00595	3.125	No hits found	837	1438	1569	357	467	371
contig04735	0.01886	3.123	gi|197342428|ref|ZP_03180248.1| acetyltransferase [*Streptomyces* ... 34	1048	1249	1738	264	646	438
contig02417	0.02132	3.119	gi|47206961|emb|CAF90782.1| unnamed protein product [*Tetraodon n*... 88	664	300	250	106	113	137
contig13047	0.00494	3.100	No hits found	3254	1913	1743	778	721	649
contig13158	0.04495	3.097	gi|5835683|ref|NP_008499.1|COX2_15044 cytochrome c oxidase subun... 215	55535	28781	41236	6610	19898	16874
contig08115	0.03025	3.095	gi|21307827|gb|AAL47188.1| putative odorant receptor Or5 [*Anophe*... 33	150	157	161	26	82	60
contig03384	4.1E-04	3.089	No hits found	178	212	200	69	53	70
contig01481	0.02657	3.081	gi|194224697|ref|XP_001495027.2| PREDICTED: similar to fructose-... 89	350	479	1031	173	207	165
contig11204	0.04833	3.080	No hits found	873	426	452	331	161	108
contig00725	0.04163	3.075	gi|195396001|ref|XP_002056621.1| GJ11045 [*Drosophila virilis*] >g... 99	193	244	532	61	113	125
contig11786	0.04683	3.075	gi|67517927|ref|XP_658738.1| QUTA_EMENI QUINIC ACID UTILIZATION ... 33	1610	526	675	192	388	264
contig03915	0.04789	3.061	No hits found	154	246	208	32	113	76
contig12655	0.02816	3.051	gi|171688916|ref|XP_001909398.1| unnamed protein product [*Podosp*... 42	553	439	385	131	270	93
contig12306	0.00259	3.043	gi|39653771|ref|NP_945222.1| NADH dehydrogenase subunit 5 [*Haema*... 125	30010	22941	36701	7970	10556	10662
contig11947	0.00953	3.035	No hits found	179	237	236	53	109	62
contig13316	0.03218	3.006	gi|170089261|ref|XP_001875853.1| predicted protein [*Laccaria bic*... 35	209	371	592	111	162	94
contig06979	0.03059	-3.056	No hits found	806	1402	1309	1938	4714	4621
contig07485	0.04292	-3.249	No hits found	180	183	123	250	614	905
contig06768	0.03542	-3.262	gi|70909867|emb|CAJ17420.1| ribosomal protein L35Ae [*Carabus gra*... 164	46	27	28	209	75	77
contig11750	0.03351	-3.499	gi|124487974|gb|ABN12070.1| putative RNA-dependent helicase p68 ... 139	1675	2180	2432	3517	8590	12593
contig12184	0.04160	-3.505	gi|149921489|ref|ZP_01909941.1| hypothetical protein PPSIR1_3079... 33	35	23	12	100	42	99
contig13133	0.04525	-3.529	gi|67083339|gb|AAY66605.1| putative salivary protein [*Ixodes sca*... 41	2490	2121	4154	4541	15004	14154
contig00109	0.02191	-3.552	gi|194222651|ref|XP_001497265.2| PREDICTED: eIF4GI protein [*Equu*... 48	14	13	36	71	53	78
contig13213	0.04280	-3.692	gi|195112951|ref|XP_002001035.1| GI22201 [*Drosophila mojavensis*]... 37	86	71	35	110	342	286
contig13166	0.01018	-3.708	No hits found	1375	831	662	2556	3125	4828
contig13029	0.02695	-4.148	gi|188572677|ref|YP_001911141.1| hypothetical protein CBPV_s2gp3... 48	1345	1757	2295	3412	12174	9319

Probe	p-value	Fold change Fed vs Unfed	Gene	F1	F2	F3	S1	S2	S3
contig13326	0.01507	-4.307	gi|148255061|ref|YP_001239646.1| putative ABC transporter (perme... 35	538	667	1024	1694	4528	3828
contig13325	0.02267	-4.322	No hits found	442	261	173	1743	1427	648
contig00150	0.03440	-4.391	gi|91081773|ref|XP_973360.1| PREDICTED: similar to GA13521-PA [T... 82	106	259	213	378	960	1364
contig00603	0.00303	-4.429	gi|77553801|gb|ABA96597.1| MYB10, putative, expressed [*Oryza sat*... 36	849	921	954	2547	4826	5273
contig00416	0.01391	-4.673	gi|67603978|ref|XP_666583.1| hypothetical protein Chro.70593 [*Cr*... 39	2990	5869	3816	10185	25123	26701
contig13119	0.00935	-4.703	gi|116059360|emb|CAL55067.1| unnamed protein product [*Ostreococc*... 35	23	11	9	51	86	54
contig07154	0.00301	-4.765	No hits found	36	49	42	193	138	301
contig04870	0.00218	-5.137	gi|183979376|dbj|BAG30740.1| muscle myosin heavy chain [*Papilio* ... 244	19	37	31	181	120	136
contig13174	0.01353	-5.251	gi|171678553|ref|XP_001904226.1| unnamed protein product [*Podosp*... 35	705	297	428	1306	2765	3594
contig13310	0.02038	-6.204	gi|16877407|gb|AAH16950.1| KLHL23 protein [*Homo s*apiens] 27	1846	451	461	3360	5534	4930
contig13245	0.02973	-6.666	gi|30023940|ref|NP_835282.1| polymerase [Indian peanut clump virus] 39	47	16	28	89	458	153
contig13337	0.01148	-6.932	No hits found	1546	541	406	3575	5072	6239
contig13361	0.01738	-7.228	gi|145598291|ref|YP_001162367.1| hypothetical protein YPDSF_0994... 33	1558	1869	1275	4171	18438	18228
contig00606	7.1E-04	-32.170	gi|189242145|ref|XP_001813277.1| PREDICTED: similar to polyprote... 70	3212	19 15	2612	149149	7682 0	46685

Appendix 4:

Published paper